PATENT
Attorney Docket No.: AVT-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Wood et al.	CONF. NO:	8540
APPLICATION NO.:	10/068,299	GROUP NO:	1651
FILING DATE:	02/06/2002	EXAMINER:	Barnhart, Lora Elizabeth
TITLE:	CELL SUSPENSION PREPARATION TECHNIQUE AND DEVICE		

Mail Stop RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CONTINUED EXAMINATION AFTER FILING NOTICE OF APPEAL AMENDMENT AND REMARKS

Sir:

Following the filing of a Notice of Appeal on March 23, 2009, Applicants hereby submit this Request for Continued Examination and Amendment and Remarks (the "Request and Amendment") in response to the final Office Action mailed from the U.S. Patent Office on September 23, 2008. Applicants respectfully request an extension of time for filing the Request and Amendment up to October 23, 2009, and hereby authorize the Commissioner to charge the requisite fees due in connection with this Request and Amendment to Deposit Account No. 50-3081.

Applicants hereby request Continued Examination of the present application pursuant to 37 C.F.R. §1.114 and enclose claim amendments together with remarks regarding the patentability of the amended claims.

Applicants respectfully request entry of this Amendment and Remarks, in which:

- **Claim Amendments** begin on page 2.
- **Remarks** begin on page 9.

Amendments to the Claims

This listing of the claims will replace all prior versions and listings of the claims in the application.

Listing of Claims

1-28. (Canceled)

29. (Currently amended) A cell suspension produced according to a method comprising the steps of:

(a) physically and/or chemically dissociating cellular stratum in a dermal-epithelial tissue sample obtained from a patient to provide cells suitable for grafting to [[a]] the patient;

(b) harvesting the cells in the presence of a nutrient solution, the harvested cells having the potential to include cellular congregates ~~cell conglomerates~~; and

(c) filtering the cells in nutrient solution to remove cellular congregates greater than 200 μM ~~cell conglomerates~~,

wherein the resulting cell suspension is free of ~~xenogenic~~ serum xenogenic to said patient and of cellular congregates greater than 200 μM ~~cell conglomerates the cells remain viable, and the suspension is suitable for direct application to a region on a patient undergoing tissue grafting~~, and

wherein the suspension comprises a composition of viable cells autologous to said patient, and wherein said composition has a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in said tissue sample.

30-33. (Canceled)

34. (Withdrawn) A cell suspension according to claim 29, the suspension being distributed on a patient tissue site undergoing tissue grafting.

35. (Withdrawn) A cell suspension according to claim 29 comprising the further step of:

(d) administering the suspension directly to a region on the patient that requires a cell graft.

36. (Withdrawn) A cell suspension according to claim 35 wherein the tissue sample is obtained from the patient that requires a cell graft.

37. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is distributed relatively evenly over the graft region.

38. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively.

39. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension contains cells present in a ratio to each other comparable to those in the donor sample.

40. (Withdrawn) A cell suspension according to claim 39 wherein the cells include keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

41. (Withdrawn) A suspension according to claim 40 wherein the cells are substantially viable.

42. (Withdrawn) A cell suspension according to claim 37 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

43. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively from a tissue sample for the patient that requires a cell graft, contains cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

44. (Withdrawn) A cell suspension produced according to a method comprising the steps of:

(a) obtaining a tissue sample from a site on a donor in need of a tissue graft;

(b) physically and/or chemically dissociating and removing cellular stratum from cells present in the sample;

(c) harvesting the cells in the presence of a nutrient solution;

(d) distributing the suspension on a site of the donor as an autologous tissue graft.

45. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of cell conglomerates.

46. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of xenogenic serum.

47. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is distributed relatively evenly over the graft region.

48. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is obtained perioperatively.

49. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension contains cells represent in a ratio to each other comparable to those seen in the donor sample.

50. (Withdrawn) A suspension according to claim 49 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

51. (Withdrawn) A suspension according to claim 50 wherein the cells are substantially viable.

52. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is sprayed, spread, pipetted, or pained on to the tissue site.

53. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is obtained perioperatively, contains cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

54. (Withdrawn) A cell suspension produced by a method comprising obtaining cells from a patient in need of a tissue graft, providing the cells in nutrient solution in a manner that is substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension being distributed in apposition to the site of the recipient as a tissue graft.

55. (Withdrawn) A suspension according to claim 54 wherein the suspension is distributed relatively evenly over the graft region.

56. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension is obtained perioperatively.

57. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

58. (Withdrawn) A suspension according to claim 57 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

59. (Withdrawn) A suspension according to claim 58 wherein the cells are substantially viable.

60. (Withdrawn) A cell suspension according to claim 55 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

61. (Currently amended) A cell suspension comprising cells derived from a dermal-epithelial tissue sample obtained from a patient, ~~site of a donor and produced by a method that provides a~~ the cell suspension comprising:

(a) a composition of viable cells derived from said tissue sample and autologous to said patient, said composition having a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in said tissue sample ~~autologous cells substantially lacking cellular stratum~~; and

(b) [the cells being provided in] a nutrient solution free of ~~xenogenic~~ serum ~~and of cell conglomerates~~ xenogenic to the patient,

wherein said cell suspension is free of cellular congregates greater than 200 μm.

~~; (c) the cells being viable, and the suspension being adapted for direct application to a region of the donor as a tissue graft, and~~

~~(d) the cell population being substantially the same as the tissue site.~~

62. (Withdrawn) A suspension according to claim 61 wherein the suspension is distributed relatively evenly over the graft region.

63. (Previously presented) A cell suspension according to claim 61 wherein the cell suspension is obtained perioperatively.

64. (Canceled)

65. (Currently amended) A suspension according to claim [[64]] 61 wherein said composition of [[the]] cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

66. (Canceled)

67. (Withdrawn) A cell suspension according to claim 62, wherein the cell suspension is sprayed, spread pipetted, or painted onto the tissue site.

68. (Withdrawn) A cell suspension produced by a method sufficient to provide the cells in nutrient solution, substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension serving as a graft in apposition to the body of a recipient in need of a tissue graft.

69. (Withdrawn) A suspension according to claim 68 wherein the suspension is distributed relatively evenly over the graft region.

70. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is obtained perioperatively.

71. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

72. (Withdrawn) A suspension according to claim 71 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

73. (Withdrawn) A suspension according to claim 72 wherein the cells are substantially viable.

74. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

75. (New) A cell suspension according to claim 61, further comprising an enzyme.

76. (New) A cell suspension according to claim 75 wherein the enzyme is selected from the group consisting of trypsin, trypsin-edta, dispase, collagenase, thermolysin, pronase, hyaluronidase, pancreatin, elastase, and papain.

77. (New) A cell suspension according to claim 76 wherein the enzyme is trypsin.

78. (New) A cell suspension according to claim 77 wherein the trypsin is present in a solution in an amount that is between 5 and 0.1% per volume of the solution.

79. (New) A cell suspension according to claim 77 wherein the trypsin is present in a solution in an amount that is less than 0.05% per volume of the solution.

REMARKS

I. CLAIM AMENDMENTS

Claims 29 and 34-74 were considered in the final Office Action of September 23, 2008. Claims 34-60, 62 and 67-74 stand withdrawn from consideration as allegedly being directed to a non-elected invention, and claims 29, 61 and 63-66 stand rejected. Upon entry of this Request and Amendment, claims 29 and 34-63, 65, 67-78 will be pending in the application.

Claims 29 and 61 are amended herein to clarify that the tissue sample is a dermal-epithelial sample obtained from a patient, and that the cell suspension is free of serum xenogenic to the patient and of cellular congregates greater than 200 μM. Claims 29 and 61 are also amended to provide that the suspension comprises a composition of viable cells autologous to the patient and having a comparable ratio of cell types. Claim 65 is amended for consistency. New claims 75-79 are added. Finally, claims 64 and 66 are canceled herein.

Support for the foregoing amendments and new claims is found in the application as filed at least at page 4, lines 10-16; page 8, lines 18-24; page 9 lines 24-30, page 10, lines 4-9 and lines 22-23; page 11, lines 8-25; and page 12, line 23-30. No new matter has been introduced by the amendments made herein.

II. RESPONSE TO REJECTIONS

In the September 23, 2008 Office Action, the Examiner rejected all of the claims (claims 29, 61 and 63-66) under 35 U.S.C. § 112, second paragraph. In addition, the Examiner rejected all of the claims under 35 U.S.C. § 102 over one or more of the following references:

1. U.S. Patent No. 4,418,691 to Yannas et al. ("**Yannas**")
2. European Patent Application No. 0 350 887 to Suzuki et al. ("**Suzuki**")
3. Hirobe, *Journal* of *Cellular Physiology* 152: 337-345, 1992 ("**Hirobe**")
4. Noel-Hudson et al., *In Vitro Cell and Developmental Biology- Animal* 31: 508-515, 1993 ("**Noel-Hudson**")
5. U.S. Patent No. 5,328,695 to Lucas et al. ("**Lucas**")
6. U.S. Patent No. 5,556,783 to Lavker et al. ("**Lavker**")
7. U.S. Patent No. 5,786,207 to Katz et al. ("**Katz**")
8. Osborne et al., *Biomaterials* 20: 283-290, 1999 ("**Osborne**")
9. U.S. Patent No. 6,207,451 to Dennis et al. ("**Dennis**")

In an effort to expedite prosecution of the application, Applicants have amended certain claims and canceled others. In making these amendments, Applicants are not acquiescing to the pending rejections and are not abandoning or surrendering any of the subject matter in previous versions or listings of the claims or in the application. Accordingly, Applicants reserve the right to pursue claims of similar, narrower, or broader scope in the future.

In view of the amendments to the claims and the following remarks, Applicants respectfully request reconsideration and withdrawal of the rejections made in the outstanding Office Action.

A. Rejections Under 35 U.S.C. §112, Second Paragraph

The Examiner rejected claims 29, 61, and 63-66 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded by the Applicants as the invention. Specifically, the Examiner requested clarification of "xenogenic serum" in claims 29 and 61, and "substantially the same as the tissue site" in claim 61. The Examiner also objected to "the cell population" in claim 61 and "the donor sample" in claim 64 for lack of antecedent basis.

Claims 29 and 61 have been amended in relevant part to clarify that the cell suspension is free of serum xenogenic to the patient and that the suspension comprises a composition of viable cells autologous to the patient and having a particular ratio of cell types. Furthermore, claims 64 and 66 have been canceled. In view of the foregoing amendments, Applicants respectfully request withdrawal and reconsideration of the rejections under 35 U.S.C. §112, second paragraph are respectfully requested.

B. Rejection Under 35 U.S.C. § 102(b) Over Yannas

Independent claims 29, 61 and dependent claims 63 and 65 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 4,418,691 to Yannas et al. ("Yannas"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Yannas does not anticipate each of

claims 29, 61, and 63-66, at least because Yannas does not disclose each and every element of independent claims 29 and 61.

Specifically, Yannas does not teach or suggest a cell suspension that is *both* free of serum xenogenic to the patient *and* free of cellular congregates greater than 200 μm as required by Applicants' amended claims 29 and 61. By contrast, the cell suspension disclosed by Yannas necessarily contains serum xenogenic to the patient because the cells are treated in tissue culture medium "supplemented with 10% fetal calf serum." Yannas at column 16, lines 10-15. Yannas further reports filtering the serum-supplemented suspension, but only through sterile gauze. Yannas specifically states that the purpose of filtering is "to remove ***large tissue fragments***." Yannas at column 16, lines 18-19, emphasis added. Sterile gauze is well-known in the art as a loose, woven fabric. While it has many uses, it is in no way effective for filtering down to the 200 μm size. As such, the cell suspension of Yannas is not free of serum xenogenic to the patient nor free of cellular congregates greater than 200 μm, as recited by claims 29 and 61 of the instant invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of this 35 U.S.C. § 102(b) rejection of claims 29 and 61, as well as claims 63 and 65 that depend therefrom.

B. Rejection Under 35 U.S.C. § 102(b) Over Suzuki

Independent claims 29, 61 and dependent claim 63 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by European Patent Application No. 0 350 887 to Suzuki et al. ("Suzuki"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Suzuki does not anticipate each of claims 29 and 61 at least because Suzuki does not disclose each and every element of independent claims 29 and 61.

Specifically, Suzuki fails to teach or suggest cells from a dermal-epithelial tissue sample as required by Applicants' amended claims 29 and 61. Instead, as the Examiner acknowledges, Suzuki teaches cells dissociated from heart tissues. Suzuki at page 5, lines 50-54. See also Office Action at page 10, first paragraph. Because heart only includes mesothelial, endotheliual and myocardial cells, the cell suspension of Suzuki excludes cells from dermal-epithelial tissues.

For the same reason, Suzuki does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, nor a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in the tissue sample as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Suzuki. Claim 63 is dependent upon claim 61, and thus is also patentable over Suzuki. Applicants respectfully request that the rejection of claims 29, 61 and 63 under 35 U.S.C. §102(b) over Suzuki be reconsidered and withdrawn.

C. Rejection Under 35 U.S.C. § 102(b) Over Hirobe

Independent claims 29, 61 and dependent claims 63 and 65 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hirobe, *Journal* of *Cellular Physiology* 152: 337-345, 1992 ("Hirobe"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Hirobe does not anticipate each of claims 29, 61, 63 and 65, at least because Hirobe does not disclose each and every element of independent claims 29 and 61.

Specifically, Hirobe does not disclose at least three of Applicants' claimed elements: (1) a cell suspension made from a dermal-epithelial tissue sample obtained from a patient, wherein the suspension is free of serum that is xenogenic to the patient; (2) a composition of cells autologous to a patient, and (3) a composition of cells having a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in the tissue sample obtained from the patient. Instead, the cell suspension reported by Hirobe comprises a mixed population of cells derived from the whole skin of newborn mice in serum-containing medium that is then subjected to melanocyte- or keratinocyte-defined medium to produce a cell population that is dominated by melanocytes or keratinocytes. Hirobe at page 337, left column, third paragraph. See also Office Action at page 11, second paragraph. Hirobe completely fails to teach a cell suspension that comprises a cell population having a ratio of keratinocytes, fibroblasts and melanocytes that is comparable to the ratio present in a sample taken from a patient, *and* that is free of serum that is xenogenic to the

patient from which it the cells are derived. As such, Hirobe fails to teach each and every limitation of the instant claims.

For at least the foregoing reasons, claims 29 and 61 are patentable over Hirobe. Claims 63 and 65 are dependent upon claim 61, and thus are also patentable over Hirobe. Applicants respectfully request that the rejection of claims 29, 61, 63 and 65 under 35 U.S.C. §102(b) over Hirobe be reconsidered and withdrawn.

D. Rejection Under 35 U.S.C. § 102(b) Over Noel-Hudson

Independent claims 29, 61 and dependent claim 63 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Noel-Hudson et al., *In Vitro Cell and Developmental Biology- Animal* 31: 508-515, 1993 ("Noel-Hudson"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Noel-Hudson does not anticipate each of claims 29, 61 and 63, at least because Noel-Hudson does not disclose each and every element of independent claims 29 and 61.

Specifically, Noel-Hudson does not teach or suggest a cell suspension that is free of serum xenogenic to the patient as required by Applicants' amended claims 29 and 61. By contrast, Noel-Hudson's cell suspension necessarily contains serum xenogenic to the patient, because the cells are cultured in media containing 5% fetal calf serum. Noel-Hudson at Abstract and at page 509, left column, seventh paragraph.

Furthermore, Noel-Hudson fails to teach or suggest cells from a dermal-epithelial tissue sample or a composition that includes keratinocyte basal cells, fibroblasts and melanocytes. Instead, as the Examiner acknowledges, Noel-Hudson teaches cells obtained from human foreskin. Noel-Hudson at page 509, right column, paragraph 7. See also Office action at page 12, second paragraph. In addition, Noel-Hudson teaches cell suspensions of keratinocytes in the absence of fibroblasts. Noel-Hudson at Abstract. Thus Noel-Hudson fails to disclose a composition having keratinocyte basal cells, fibroblasts and melanocytes, and also fails to disclose a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in the tissue sample as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Noel-Hudson. Claim 63 is dependent upon claim 61, and thus is also patentable over Noel-Hudson. Applicants respectfully request that the rejection of claims 29, 61 and 63 under 35 U.S.C. §102(b) over Noel-Hudson be reconsidered and withdrawn.

E. Rejection Under 35 U.S.C. § 102(b) Over Lucas

Independent claims 29, 61 and dependent claim 63 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,328,695 to Lucas et al. ("Lucas"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Lucas does not anticipate each of claims 29, 61 and 63 at least because Lucas does not disclose each and every element of independent claims 29 and 61.

Specifically, Lucas fails to teach or suggest cells from a dermal-epithelial tissue sample obtained from a patient. The Examiner points to Lucas at Example 5 (column 11, lines 11-25) for skin tissue. Office action at page 14, second paragraph. However, the tissues used by Lucas are from white Leghorn chick embryos. Lucas at column 11, lines 1-4. Furthermore, Lucas does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, and also fails to disclose a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in a tissue sample obtained from a patient as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Lucas. Claim 63 is dependent upon claim 61, and thus is also patentable over Lucas. Applicants respectfully request that the rejection of claims 29, 61 and 63 under 35 U.S.C. §102(b) over Lucas be reconsidered and withdrawn.

F. Rejection Under 35 U.S.C. § 102(b) Over Lavker

Independent claims 29, 61 and dependent claims 63 and 65 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,556,783 to Lavker et al. ("Lavker"). In order for a claim to be anticipated each and every element of the claim must be present in the

cited art. Applicants respectfully submit that Lavker does not anticipate each of claims 29, 61, 63 and 65, at least because Lavker does not disclose each and every element of independent claims 29 and 61.

Specifically, Lavker does not teach or suggest a cell suspension that is *both* free of serum xenogenic to the patient *and* free of cellular congregates greater than 200 μm as required by Applicants' amended claims 29 and 61. By contrast, Lavker's cell suspension necessarily contains serum xenogenic to the patient, because the cells are treated in tissue culture medium "supplemented with 17% fetal calf serum." Lavker at column 8, lines 35-40. Furthermore, Lavker does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, nor a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in a tissue sample obtained from a patient as claimed by Applicants. Rather, Lavker discloses follicular keratinocytes. Lavker, column 5, lines 29-44.

For at least the foregoing reasons, claims 29 and 61 are patentable over Lavker. Claims 63 and 65 are dependent upon claim 61, and thus are also patentable over Lavker. Applicants respectfully request that the rejection of claims 29, 61, 63 and 65 under 35 U.S.C. §102(b) over Lavker be reconsidered and withdrawn.

G. Rejection Under 35 U.S.C. § 102(b) Over Katz

Independent claims 29, 61 and dependent claim 63 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,786,207 to Katz et al. ("Katz"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Katz does not anticipate each of claims 29, 61 and 63, at least because Katz does not disclose each and every element of independent claims 29 and 61.

Specifically, Katz fails to teach or suggest cells from a dermal-epithelial tissue sample obtained from a patient. By contrast, Katz only teaches a general method for tissue dissociation and producing a cellular suspension. Katz at Abstract. Furthermore, Katz does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, and also fails to

disclose a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in a tissue sample obtained from a patient as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Katz. Claim 63 is dependent upon claim 61, and thus is also patentable over Katz. Applicants respectfully request that the rejection of claims 29, 61 and 63 under 35 U.S.C. §102(b) over Katz be reconsidered and withdrawn.

H. Rejection Under 35 U.S.C. § 102(b) Over Osborne

Independent claims 29, 61 and dependent claims 63 and 65 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Osborne et al., *Biomaterials* 20: 283-290, 1999 ("Osborne"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Osborne does not anticipate each of claims 29, 61, and 63 and 65, at least because Osborne does not disclose each and every element of independent claims 29 and 61.

Specifically, Osborne fails to teach or suggest cells from a dermal-epithelial tissue sample as required by Applicants' amended claims 29 and 61. In direct contrast, Osborne teaches isolating keratinocytes by mincing the epidermis and isolating fibroblasts by mincing the dermis. Osborne at page 284, Section 2.3. Osborne's cell suspensions thus have isolated cell types: keratinocyte or fibroblast (but not combined). For the same reason, Osborne does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, and also does not disclose a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in the tissue sample as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Osborne. Claims 63 and 65 are dependent upon claim 61, and thus are also patentable over Osborne. Applicants respectfully request that the rejection of claims 29, 61, and 63 and 65 under 35 U.S.C. §102(b) over Osborne be reconsidered and withdrawn.

I. Rejection Under 35 U.S.C. § 102(b) Over Dennis

Independent claims 29, 61 and dependent claim 63 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 6,207,451 to Dennis et al. ("Dennis"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Dennis does not anticipate each of claims 29, 61, and 63 at least because Dennis does not disclose each and every element of independent claims 29 and 61.

Specifically, Dennis fails to teach or suggest cells from a dermal-epithelial tissue sample or a composition that includes keratinocyte basal cells, fibroblasts and melanocytes. Instead, as the Examiner acknowledges, Dennis teaches cells dissociated from muscle tissue from which skin has been removed. Dennis at column 12, lines 13-17. See also Office action at page 19, second paragraph. Accordingly, Dennis does not disclose a composition that includes keratinocyte basal cells, fibroblasts and melanocytes, and also does not disclose a ratio of keratinocyte basal cells, fibroblasts and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in the tissue sample as claimed by Applicants.

For at least the foregoing reasons, claims 29 and 61 are patentable over Dennis. Claim 63 is dependent upon claim 61, and thus is also patentable over Dennis. Applicants respectfully request that the rejection of claims 29, 61 and 63 under 35 U.S.C. §102(b) over Dennis be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that the claims, as amended, are in condition for allowance and request favorable action. The Examiner is invited to contact Applicants' attorney at the number below if in the Examiner's view it would expedite the examination of the application.

The Commissioner is hereby authorized to charge any fee occasioned by the entry of this paper to Attorney's Deposit Account No. 50-3081.

Respectfully submitted,

October 19, 2009
Date

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Exhibit B

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/068,299	02/06/2002	Fiona M. Wood	AVT-001	8540

42532 7590 12/30/2009

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BARNHART, LORA ELIZABETH	
ART UNIT	**PAPER NUMBER**
1651	

MAIL DATE	DELIVERY MODE
12/30/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/068,299	WOOD ET AL.	
	Examiner	Art Unit	
	Lora E. Barnhart	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 October 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *29,34-63,65 and 67-79* is/are pending in the application.

4a) Of the above claim(s) *34-60,62 and 67-74* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *29,61,63,65 and 75-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/19/09 has been entered.

Response to Amendments

Applicant's amendments filed 10/19/09 to claims 29, 61, and 65 have been entered. Claims 64 and 66 have been canceled. Claims 75-79 have been added. Claims 29, 34-63, 65, and 67-79 remain pending in the current application, of which claims 29, 61, 63, 65, and 75-79 are being considered on their merits. Claims 34-60, 62, and 67-74 remain withdrawn from consideration at this time. References not included with this Office action can be found in a prior action. Any rejections of record not particularly addressed below are withdrawn in light of the claim amendments and applicant's comments.

The amendment to claim 61 is not fully compliant with 37 C.F.R. 1.121(c). Specifically, the matter in brackets in step (b) should be struck-through, not placed in brackets; five or fewer consecutive characters may be deleted by enclosing them in double brackets. However, in the interest of compact prosecution, and considering applicant's description of the amendments at page 9 of the remarks, the examiner

agrees to consider the claims on their merits, interpreting the phrase "the cells being provided in" as being deleted from step (b) of claim 61. **Future submissions that do not comply fully with 37 C.F.R. 1.121 will be considered nonresponsive.**

Claim Objections

Claim 65 is objected to because of the following informalities: It should read, "wherein said composition of cells comprise**s** keratinocyte …" to comply with standard English. Appropriate correction is required.

Claim 76 is objected to because of the following informalities: It should read, "trypsin-EDTA," i.e. "EDTA" in all capital letters. Correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 29, 61, 63, 65, and 75-79 are/remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "dermal-epithelial" in claims 29 and 61 is queried. Dermal tissue inherently contains epithelium, and the specification refers to the "dermal-epithelial junction" (page 11, lines 12-20). It is not clear whether the claim term refers simply to any dermal sample such as the biopsy obtained in the working example at page 21, lines 8-14, or whether it is necessarily limited to the cells at the dermal-epithelial junction (such as those obtained at page 22, lines 10-14, of the specification). Clarification is required.

Claims 29 and 61 also recite a cell composition "having a ratio of keratinocyte basal cells, fibroblasts, and melanocytes that is comparable to a ratio of keratinocyte basal cells, fibroblasts, and melanocytes present in [a patient or a tissue sample from which the population is obtained]," which is confusing. First, a ratio is, by definition, a comparison of two numbers, so the meaning of a ratio of three values is not clear. Second, in reciting "a ratio" (as opposed to "the ratio"), the term does not limit the claimed ratio to any particular one, e.g. the ratio of the total number of one cell type to the total number of another cell type within the population. Third, the criteria for determining whether one ratio is "comparable" to another are not clear; all numbers are literally comparable to each other in that it is possible to compare them. Clarification is required.

Claim 61 requires "a composition of cells derived from said tissue sample," but the word "derived" does not clearly set forth the relationship between the cells and the sample. It is not clear to what degree, if any, the tissue may be manipulated and still yield cells "derived" from it. The claim does not, for example, require that the composition contain both dermal and epithelial cells, since epithelial cells are "derived" from a tissue sample containing both dermis and epidermis. Clarification is required.

Because claims 63, 65, and 75-79 depend from indefinite claim 61 and do not clarify these points of confusion, they must also be rejected under 35 U.S.C. 112, second paragraph.

Claims 78 and 79 require that the trypsin in the composition be "present in a solution in an amount that is between 5 and 0.1% per volume of the solution," but it is

not clear whether this limitation addresses the concentration of trypsin in the solution that is added to the suspension to yield the composition or whether it addresses the final concentration of trypsin in the claimed composition. The as-filed specification implies that the former is the case (page 21, lines 20-25); nowhere does the specification address the final concentration of trypsin in the finished cell composition. The claim should clearly limit the components of the claimed product.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 29, 61, 63, 65, and 75-79 are rejected under 35 U.S.C. 102(b) as being anticipated by Noel-Hudson et al. (1993, *In Vitro Cell and Developmental Biology – Animal* 31: 508-515; reference C6 on 6/1/04 IDS) taken in light of Van Bossuyt (1999, U.S. Patent 5,866,167; reference A). The claims are interpreted as being drawn to a composition comprising cells, said cells having been dissociated from some tissue, and a nutrient solution, said composition lacking large aggregates of cells. In some dependent claims, the cells are obtained during the course of a surgical operation. In some dependent claims, the composition further comprises an enzyme, e.g. trypsin.

Noel-Hudson et al. teach a composition comprising cells dissociated from human foreskin tissue biopsy fragments with 0.25% trypsin and then with a solution comprising 0.025% trypsin (page 509, column 1, paragraph 7). Noel-Hudson's composition lacks

all cell aggregates of any size ("individual cells;" *ibid.*) and comprises a physiological saline, specifically Hanks' solution with calcium salts (*ibid.*).

Van Bossuyt is cited solely as evidence that skin (such as the skin biopsy fragments of Noel-Hudson) inherently contains keratinocytes (epithelial cells), melanocytes, and Langerhans cells in the epidermis; proliferating keratinocytes at the base of the epidermis; and fibroblasts in the dermis (column 1, lines 41-53).

The Patent and Trademark Office is not equipped to conduct experimentation in order to determine whether or not applicants' cell suspension differs, and if so to what extent, from the suspension discussed in Noel-Hudson. The prior art suspension is made by dissociating skin samples with trypsin, as is the instant composition (Example 1 at page 21, et seq.); furthermore, Van Bossuyt's teachings indicate that the whole-skin biopsy of Noel-Hudson inherently contains all of the cell types recited in claims 29, 61, and 65. The cited art taken as a whole demonstrates a reasonable probability that the suspension of the prior art is either identical or sufficiently similar to the claimed suspension that whatever differences exist are not patentably significant. Therefore, the burden of establishing novelty or unobviousness by objective evidence is shifted to applicants.

The fact that Noel-Hudson does not specifically discuss the presence of the cell types recited in claims 29, 61, and 65 in their suspension does not make that suspension patentable. Applicant's suspension possesses inherent characteristics which might not be displayed in the tests used in Noel-Hudson; it is noted that all of the cell types recited in claims 29, 61, and 65 are inherently present in skin tissue. Clear

evidence that the suspension of the cited prior art does not possess a critical characteristic that is possessed by the claimed suspension (e.g., the presence of all of the recited cell types) would advance prosecution and might permit allowance of claims to applicants' suspension.

Claim 29 is a product-by-process claim. M.P.E.P. § 2113 reads, "Product-by-process claims are not limited to the manipulations of the recited steps, only the structure implied by the steps." **Once a product appearing to be substantially identical is found and an art rejection made, the burden shifts to the applicant to show an unobvious difference**. In this case, this rejection might be overcome by a substantive evidentiary showing that the method steps recited in the cited claims produce a composition that is materially and patentably distinct from the skin cell suspension of Noel-Hudson.

Applicants state that Noel-Hudson does not teach all of the limitations of the claimed compositions because Noel-Hudson's composition allegedly contains calf serum. These arguments have been fully considered, but they are not persuasive. Applicant has mischaracterized the teachings of Noel-Hudson. The paragraph relied upon by the examiner reads:

> *Cells and culture conditions.* Human keratinocytes were isolated from human foreskin of [a] 1-yr-old donor, as described by Boyce and Ham (7). Briefly, the biopsy fragments were first treated with 0.25% trypsin (wt/vol) and 1000 U/ml collagenase (wt/vol) in Hanks' solution containing Ca++ for 2 h at 37° C, then with a 0.025% trypsin (wt/vol): 0.01% EDTA (wt/vol) solution to release individual cells.

The procedure discussed in this paragraph yields a suspension of foreskin tissue (and, therefore, all cell types inherently present therein) in a calcium solution containing trypsin and lacking any serum whatsoever. The fact that Noel-Hudson subjected their suspension to further culturing steps is irrelevant here. The suspension yielded by the method in this paragraph anticipates or renders obvious the claimed suspension.

Claims 29, 61, 63, and 75-79 are rejected under 35 U.S.C. 102(b) as being anticipated by Hirobe (1991, *Journal of Experimental Zoology* 257: 184-194; reference U). The claims are interpreted as being drawn to a composition comprising cells, said cells having been dissociated from some tissue, and a nutrient solution, said composition lacking large aggregates of cells. In some dependent claims, the cells are obtained during the course of a surgical operation. In some dependent claims, the composition comprises an enzyme, e.g. trypsin.

Hirobe teaches a composition comprising cells dissociated from mouse whole skin tissue by cutting the tissue into small pieces and incubating the pieces tissue in a 0.25% solution of trypsin (page 185, under “Culture of melanocytes”). The composition of Hirobe is a suspension of single cells (*Id.*). and comprises a serum-free physiological saline, specifically melanoblast defined medium, which comprises salts (*Id.*).

Claim 29 is a product-by-process claim. Once a product appearing to be substantially identical is found and an art rejection made, the burden shifts to the applicant to show an unobvious difference. In this case, this rejection might be overcome by a substantive evidentiary showing that the method steps recited in the

cited claims produce a composition that is materially and patentably distinct from the skin cell suspension of Hirobe. See M.P.E.P. § 2113. The discussion of inherent properties in the rejection over Noel-Hudson also applies to this rejection for similar reasons.

Regarding the previous art rejection over Hirobe (1992, *Journal of Cellular Physiology* 162: 337-345), applicant alleges that the composition does not contain the ratios of cells instantly claimed or a composition lacking serum. See reply, page 12, last paragraph. These arguments have been fully considered, but they are not persuasive.

As discussed in the indefiniteness rejections above, the limitations regarding the "comparable" "ratios" do not particularly point out and distinctly describe the invention. The composition of Hirobe is produced from a sample that has been enzymatically dissociated; therefore, the composition contains those cells that were present in the tissue sample. There is no disclosure in Hirobe that any cells have been destroyed, so the amount of each type of cell relative to each other is comparable to that in the tissue; "comparable" is not synonymous with "identical."

Regarding the presence of serum, Hirobe clearly teaches that the cells released by the enzymatic digestion step and dissociated with the Pasteur pipette are resuspended in MGM, a serum-free medium; serum is only added when the cells are placed into a dish. The fact that Hirobe later adds serum to a serum-free composition does not negate Hirobe's teaching of that serum-free composition.

No claims are allowed. No claims are free of the art.

Applicant is requested to specifically point out the support for any amendments made to the disclosure in response to this Office action, including the claims (MPEP 714.02 and 2163.06). In doing so, applicant is requested to refer to pages and line numbers in the as-filed specification, **not** the published application. Due to the procedure outlined in MPEP § 2163.06 for interpreting claims, it is noted that other art may be applicable under 35 U.S.C. § 102 or 35 U.S.C. § 103(a) once the aforementioned issue(s) is/are addressed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lora E. Barnhart whose telephone number is 571-272-1928. The examiner can normally be reached on Monday-Thursday, 9:00am - 5:30pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G. Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Lora E Barnhart/
Primary Examiner, Art Unit 1651

Notice of References Cited	Application/Control No. 10/068,299	Applicant(s)/Patent Under Reexamination WOOD ET AL.	
	Examiner Lora E. Barnhart	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,866,167	02-1999	Van Bossuyt, Hans	424/520
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Hirobe T. 1991. Selective growth and serial passage of mouse melanocytes from neonatal epidermis in a medium supplemented with bovine pituitary extract. J Exp Zool 257: 184-194.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes	Application/Control No.	Applicant(s)/Patent under Reexamination	
	10/068,299	WOOD ET AL.	
	Examiner	Art Unit	
	Lora E. Barnhart	1651	

SEARCHED			
Class	Subclass	Date	Examiner

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
uspats, uspgpubs (see east search history)	12/21/2009	LEB

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	("4418691").PN.	US-PGPUB; USPAT	OR	OFF	2009/12/21 12:59
L2	4217	foreskin	US-PGPUB; USPAT	OR	ON	2009/12/21 13:49
L3	196	"keratinocyte basal" and langerhans and melanocyte and fibroblast	US-PGPUB; USPAT	OR	ON	2009/12/21 13:51
L4	22	2 and 3	US-PGPUB; USPAT	OR	ON	2009/12/21 13:51
L5	228	keratinocyte same langerhans same melanocyte same fibroblast	US-PGPUB; USPAT	OR	ON	2009/12/21 13:54
L6	38	2 and 5	US-PGPUB; USPAT	OR	ON	2009/12/21 13:55

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THE JOURNAL OF EXPERIMENTAL ZOOLOGY 257:184–194 (1991)

Selective Growth and Serial Passage of Mouse Melanocytes From Neonatal Epidermis in a Medium Supplemented With Bovine Pituitary Extract

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ABSTRACT Suspensions of disaggregated epidermal cells from skins of newborn C57BL/10JHir mice were plated in a growth medium that consisted of Ham's F-10 plus bovine pituitary extract (BPE), insulin, and transferrin. Fetal bovine serum (FBS) was added to the culture medium at a concentration of 4% at the time of plating. On the second day of culture, a small number of melanocytes was randomly distributed among large sheets of keratinocytes. From the third day onward, FBS was excluded from the culture medium to prevent the proliferation of keratinocytes and fibroblasts. The melanocytes began to grow preferentially, and after 12 days pure and enriched populations of melanocytes could be harvested. In the absence of the proliferation of keratinocytes and fibroblasts, melanocytes could be serially passaged in the growth medium supplemented with a conditioned medium (CM) prepared from keratinocyte-enriched cultures, namely, those at the early stages of the primary culture. FBS was added at a concentration of 1% for the first day. These results suggest that both BPE and keratinocyte CM contain growth factors required for proliferation of melanocytes.

In the mouse embryo, melanoblasts, the precursors to melanocytes, originate from the neural crest, migrate lateroventrally from day 8, and reach all regions of the body by day 12 (Rawles, '47). Melanoblasts may enter skin via the dermis, migrate therein, and secondarily invade the epidermis between days 11 and 12 of gestation (Mayer, '73). By days 13 or 14 of gestation, the melanoblasts have colonized the epidermis (Mayer, '73). Mouse epidermal melanoblasts begin the production of unmelanized melanosomes on day 14 and begin to differentiate into melanocytes, with the appearance of tyrosinase activity, on day 16 of gestation (Hirobe, '84a). Melanocytes increase in number until 3 or 4 days after birth, and then their numbers decrease (Quevedo et al., '66; Takeuchi, '68; Weiss and Zelickson, '75; Hirobe and Takeuchi, '77, '78; Hirobe, '82). Little is known about the mechanisms that regulate the proliferation of the differentiating or differentiated melanocytes in the epidermis of mouse skin. Such a study in vivo is difficult, because the differentiated melanocytes in the mouse epidermis do not divide in the dorsal skin (Hirobe, '88b) and only divide at a low rate in the ear skin (Rosdahl, '78) under normal circumstances. Pure cultures of melanocytes, serially passaged, would provide cells in sufficient numbers for such an analysis.

Although short-lived, sparse cultures of melanocytes have been prepared from mouse epidermis (Mayer and Oddis, '77; Koyama and Takeuchi, '81; Ito and Takeuchi, '81; Mayer, '82); it has proved difficult to generate pure and enriched cultures of mouse epidermal melanocytes. Pure, enriched, long-term cultures of epidermal melanocytes from newborn mouse skin were obtained for the first time by culturing suspensions of epidermal cells with a growth medium that included 12-O-tetradecanoyl-13-acetate (TPA), cholera toxin (CT), and fetal bovine serum (FBS) (Sato et al., '85). The method was originally used for generating pure and enriched cultures of human epidermal melanocytes (Eisinger and Marko, '82). TPA promotes the growth of human and mouse melanocytes in the presence of FBS. Similarly, Bennett et al. ('87) obtained a line of malenocytes from embryonic mouse skin. Tamura et al. ('87) obtained enriched cultures of mouse dermal melanocytes from newborn mice of various strains by adding TPA, isobutylmethylxan-

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thine, human placental extract, and newborn calf serum (NCS) to a culture medium. Other lines of melanocytes were obtained from mice that carried particular mutations in coat color by using TPA and FBS or NCS (Abe et al., '86; Halaban et al., '88a; Bennett et al., '89). However, neither TPA nor CT is a natural mitogen for mouse melanocytes. To overcome this problem, we initiated the present study in an attempt to culture mouse epidermal melanocytes in a medium supplemented with a natural growth factor. Such cultures would be expected to be used in our attempts to understand the regulatory mechanisms involved in the control of the growth and proliferation of mouse epidermal melanocytes in vivo.

MATERIALS AND METHODS

Mice

All animals used in this study belonged to strain C57BL/10JHir of the house mouse *Mus musculus*. They were given water and a commercial diet (Clea Japan, Tokyo, Japan) ad libitum. They were maintained at 24 ± 1°C with 40% to 60% relative humidity; 12 hours of fluorescent light were provided daily.

Culture of melanocytes

The sources of tissue for the culture of melanocytes were dorsal skins from 0.5-day-old mice. The skin was taken from the dorsolateral side of the trunk between the limbs. The samples were cleaned of subcutaneous tissues and rinsed in calcium- and magnesium-free phosphate-buffered saline (CMF-PBS) pH 7.4, and then they were cut into small pieces (5 × 5 mm^2) and incubated in a 0.25% solution of trypsin (Sigma, St. Louis, MO) in phosphate-buffered saline (PBS, pH 7.2) for 14–16 hours at 2°C. Epidermal sheets were mechanically separated from the dermis with fine forceps and floated onto a 0.02% solution of ethylenediamine-tetra-acetate (EDTA; Dojin, Kumamoto, Japan) in CMF-PBS in plastic centrifuge tubes (Corning, Corning, NY). The tubes were gently shaken and then incubated at 37°C for 15 minutes. After this incubation, tissues were gently shaken repeatedly to produce a suspension of basal cells, and the cornified sheets were removed. Then the suspension of epidermal cells was gently and repeatedly introduced into and expelled from a Pasteur pipette to generate a suspension of single cells. Cells were pelleted by centrifugation (5 min at 1,000 rpm) and resuspended in a melanocyte growth medium (MGM) consisting of Ham's F-10 (Gibco, Grand Island, NY) supplemented with 10 μg/ml of bovine pituitary extract (BPE; Collaborative Research Inc., Bedford, MA), 10 μg/ml of insulin (Ins, bovine; Sigma), 10 μg/ml of transferrin (Tf, bovine; Sigma), and the following antibiotics: penicillin (Gibco) at 100 U/ml; streptomycin (Gibco) at 100 μg/ml; gentamicin (Sigma) at 50 μg/ml; and Fungizone (Gibco) at 0.25 μg/ml. The cells in the suspension of epidermal cells were counted in a hemocytometer chamber and mixed into medium in plastic culture dishes (Lux, Naperville, IL) at initial densities of 1, 2, and 3 × 10^6 cells/35 mm dish (1.04, 2.08, and 3.12 × 10^5 cells/cm^2). Cultures were incubated at 37°C in a humidified atmosphere composed of 5% CO_2 and 95% air (pH 7.2). At the time of plating, 4% FBS (Gibco) was added. After 2 days, 4% FBS was excluded from the culture medium to prevent proliferation of keratinocytes and fibroblasts. Medium was replaced by fresh medium three times a week. After 12–14 days, pure and enriched cultures of melanocytes were harvested. In some cases, bovine hypothalamic extract (BHE; Biomedical Technologies Inc., Stoughton, MA), epidermal growth factor (EGF, from mouse salivary gland; Takara, Kyoto, Japan), acidic fibroblast growth factor (aFGF, from bovine brain; Biomedical Technologies Inc.), basic fibroblast growth factor (bFGF[1-24], synthetic peptide, Peninsula Lab., Inc., Belmont, CA), nerve growth factor (NGF, from mouse salivary gland, 2.5S; Takara), triiodothyronine (T_3; Sigma), hydrocortisone (HC; Sigma), and CT (Sigma) were added to the culture medium to test their mitogenic activity toward melanocytes.

Serial passage of mouse epidermal melanocytes

Primary cultures of mouse epidermal melanocytes were treated with a solution of 0.05% trypsin and 0.02% EDTA in CMF-PBS at 37°C for 15 minutes. After trypsinization was inhibited by the addition of 2,000 U/ml of soybean trypsin inhibitor (Sigma), the suspensions of cells were centrifuged (5 min at 1,000 rpm) and resuspended in a passage medium that consisted of MGM and conditioned medium (CM) from primary cultures. The conditioning was continued for 1 day. For routine passages, one volume of CM was mixed with two volumes of MGM. The number of cells was counted in a hemocytometer chamber. Plating densities were 2, 4, and 6 × 10^4 cells/35 mm dish (2.08, 4.16, and 6.24 × 10^3 cells/cm^2). At the time of plating, 1% FBS was added. After 1 day

1% FBS was excluded from the culture medium. Media were replaced daily by fresh media. Cultures were carried to near confluence (3–5 days) and then repassaged and maintained in the same manner.

Conditioned media

Aliquots of 3×10^6 disaggregated epidermal cells were cultured for 2 days with MGM supplemented with 4% FBS. At this stage, cultures consisted of confluent or near-confluent keratinocytes with small numbers of melanocytes. They were then provided with 1.5 ml of fresh MGM. The cells were incubated for 1 further day, and the resulting CM from multiple paired dishes was collected with a Pasteur pipette and stored in plastic centrifuge tubes at −20°C. CM was collected daily from the cultures for up to 14 days in the same manner. Prior to use, CM was thawed and filtered through a syringe filter (0.2 μm, Corning) to remove floating cells. It was then diluted with fresh MGM (one volume of CM to two volumes of MGM). Samples were stored at 2°C until use.

Assessment of the growth of melanocytes

Melanocytes in primary and passage cultures were viable and produced pigment granules except for a small number of melanocytes that died. Dead melanocytes could be distinguished morphologically from living melanocytes. The viability of melanocytes could also be confirmed by the trypan-blue exclusion test.

Growth curves were prepared, and observations of the changes in the morphology of melanocytes were made by direct counting of melanocyte on dishes by phase-contrast and brightfield microscopy. Calculations were based on average numbers of cells in ten randomly chosen microscopic fields at a magnification of ×200, each of which covered an area of 5.81×10^{-3} cm^2. The number of melanocytes estimated by this method was similar to that determined by counting cells in suspensions of disaggregated cells in a hemocytometer after treatment with trypsin and EDTA. Bipolar, tripolar, dendritic, polygonal, and epithelioid cells that contained pigment granules were all scored as melanocytes.

Reaction with L-dopa

Cultures of mouse melanocytes were fixed with 5% formalin in phosphate buffer (pH 7.3) at 2°C for 30 minutes, rinsed with distilled water, and incubated with a 0.1% solution of L-dopa (3, 4-dihydroxyphenylalanine; Wako Pure Chemical Industries, Osaka, Japan) in phosphate buffer (pH 6.8) at 37°C for 4 hours. They were then washed with distilled water, dried in air, and sealed with glycerin.

RESULTS

Growth of melanocytes in primary culture

The number of melanocytes or melanoblasts in the epidermis has been found to differ among several inbred strains of mice (Quevedo et al., '66; Gerson and Szabó, '68; Takeuchi, '68; Hirobe, '82, '84b, '87; Tamate et al., '86). To obtain enriched cultures of melanocytes, it is better to use strains of mice that possess relatively large numbers of melanocytes and melanoblasts. Thus, mice of the C57BL/10JHir strain (Hirobe, '87) were used in the present study.

Within 1 day after initiation of cultures with MGM containing 4% FBS, individual melanocytes could be seen attached to dishes. Melanocytes appeared as small bipolar, tripolar, or dendritic cells with dark cytoplasm when examined by phase-contrast microscopy, and pigment granules were visible within them by using brightfield microscopy. Melanocytes were randomly distributed among large sheets of keratinocytes. After 2 days in vitro, these melanocytes were seen to be in contact, via dendrites, with the adjacent colonies of keratinocytes (Fig. 1A). From the third day in vitro, FBS was excluded from the culture medium. After 5–9 days in vitro, the colonies of keratinocytes were clearly smaller and refractile in appearance with progressive detachment of cells, whereas the bipolar, tripolar, or dendritic melanocytes were more numerous than before (Figs. 1B, C). After 6–9 days, cells engaged in mitotic division were often observed in the dishes, which now contained only melanocytes interspersed with small foci of dying keratinocytes (Fig. 1C). Melanocytes with mitotic figures were counted directly on the dishes by phase-contrast and brightfield microscopy. The mitotic indices at this stage ranged from about 0.2% to 0.6%. By 12–14 days, the cultures were confluent or subconfluent and contained only melanocytes (Fig. 1D). Polygonal or epithelioid melanocytes were predominant at this stage. The homogeneity of the cultures obtained by this method was confirmed by the dopa reaction. The deposition of melanin was observed both in the cytoplasm and in the dendrites of the melanocytes. The purity of the cultures of melanocytes was greater than 99%.

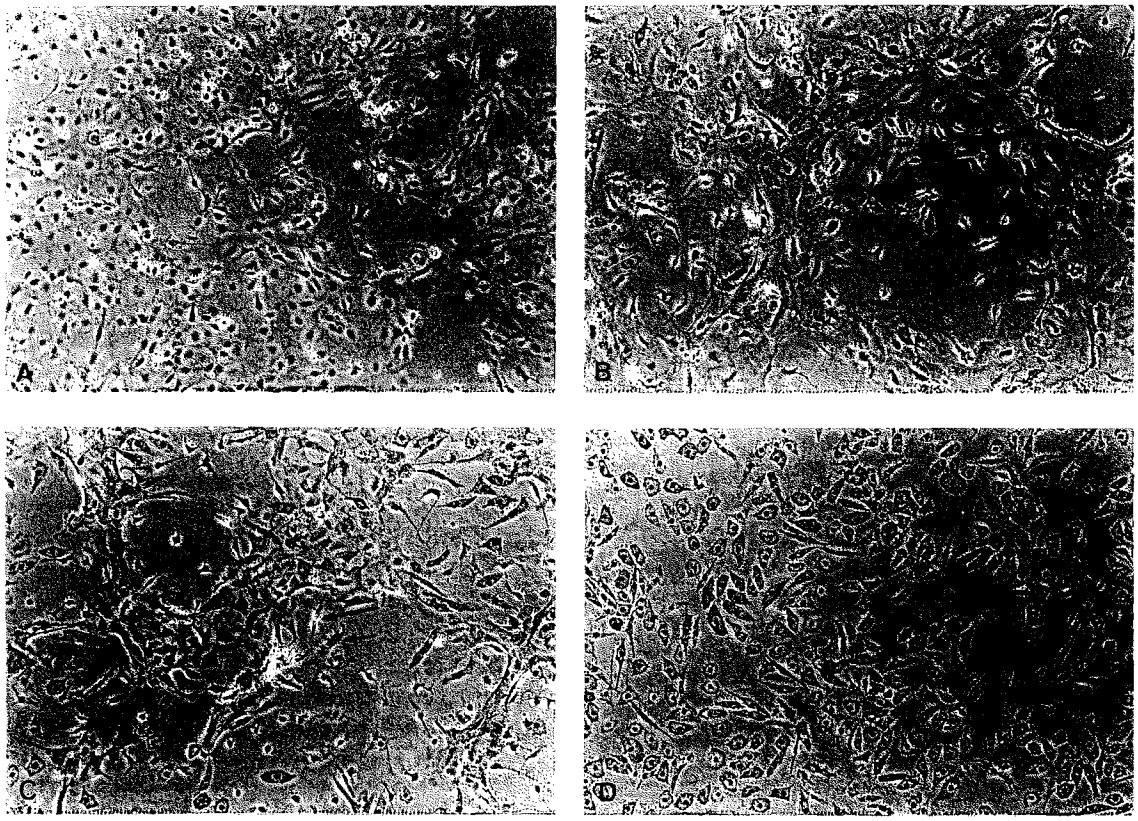

Fig. 1. Primary cultures of epidermal cells derived from mouse skin in melanocyte growth medium (MGM). Suspensions of 2×10^6 epidermal cells were plated in 35 mm dishes. Fetal bovine serum (FBS) was added to MGM to a concentration of 4% for the first 2 days. **A:** After 2 days in culture. Large colonies of keratinocytes and a small number of melanocytes (arrow) are evident. The melanocytes are bipolar, tripolar, or dendritic. **B:** After 5 days in culture. Melanocytes (arrow) have increased in number. By contrast, colonies of keratinocytes are shrinking. **C:** After 9 days in culture. Colonies of keratinocytes decrease further in size and number. By contrast, numerous melanocytes (short arrow) are seen. Long arrow indicates a mitotic melanocyte. **D:** After 12 days in culture. Enriched culture of pure melanocytes which are polygonal or epithelioid. Phase-contrast microscopy. ×60.

Melanocytes gradually decreased in number after 14 days.

Kinetics of proliferation of melanocytes in primary culture

The kinetics of proliferation of melanocytes in primary culture were studied by the direct counting of cultured melanocytes in situ under the microscope. At each of the three plating densities tested, mouse melanocytes cultured with MGM showed a logarithmic phase of growth from 2 to 9 days, followed by a stationary phase at confluence (Fig. 2). At the plating density of 3×10^6 cells/35-mm dish, the number of melanocytes observed at 12 days represented a 7.2-fold increase over the number of melanocytes at 2 days. Similar increases (5.9- and 7.2-fold) were found at the lower plating densities (2 and 1×10^6 cells/35 mm dish, respectively).

The suspensions of epidermal cells were also cultured in a medium that consisted of F-10 plus 10 μg/ml of BPE (Fig. 2). FBS was added at 4% to the culture medium for the first 2 days. Pure cultures of melanocytes were similarly obtained at the three different densities (1, 2, and 3×10^6 cells/35 mm dish) tested. However, the numbers of melanocytes observed at 12 days were about one third to one half (Fig. 2) as large as those obtained with MGM. Melanocytes increased in number slightly when the suspensions of epidermal cells were similarly cultured with a medium that consisted of F-10 plus 10 μg/ml of Ins and 10 μg/ml of Tf (Fig. 2). However, no melanocytes engaged in mitotic division were found in this case. Similar growth curves were obtained when the suspensions of epidermal cells were cultured with F-10 medium alone, with F-10 plus Ins alone, and with F-10 plus Tf alone (Table 1).

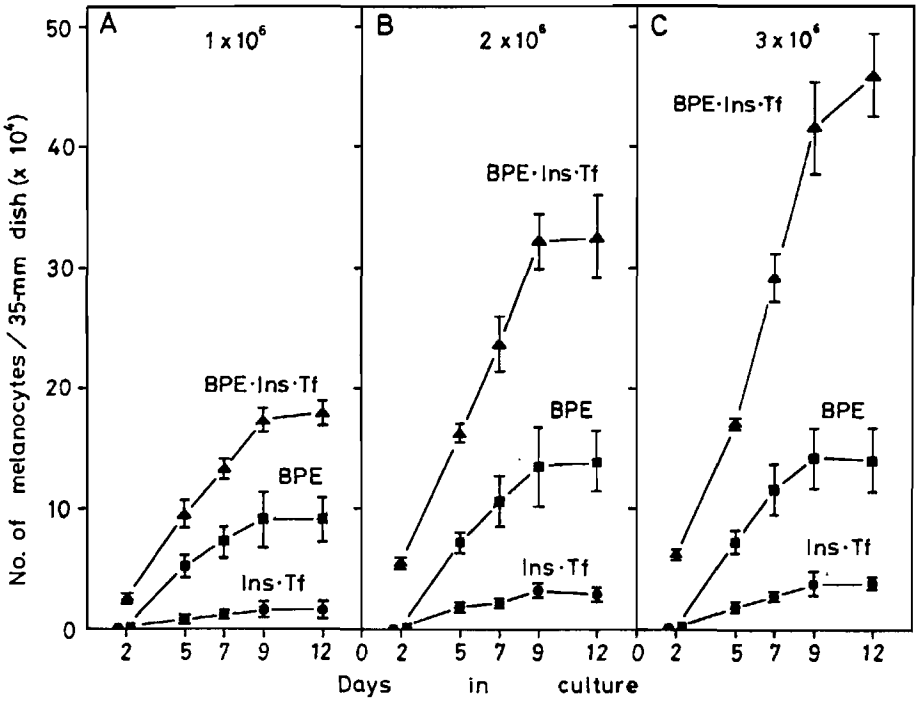

Fig. 2. Kinetics of proliferation of mouse epidermal melanocytes in primary culture. Suspensions of epidermal cells were plated in 35 mm dishes at three different densities: 1×10^6 (**A**), 2×10^6 (**B**), and 3×10^6 (**C**) cells per dish, and cultured in three different media: F-10 plus Ins and Tf at 10 μg/ml (●): F-10 plus BPE at 10 μg/ml (■); and MGM, i.e., F-10 plus BPE, Ins, and Tf at 10 μg/ml (▲). FBS was added at a concentration of 4% for the first 2 days in each case. The number of melanocytes was counted by phase-contrast and brightfield microscopy at 2, 5, 7, 9, and 12 days after plating. The cells at the three different densities were derived from the same litter of mice. The data are the averages of results of triplicate experiments with suspensions of epidermal cells from different litters of mice. Bars indicate standard errors of the means.

TABLE 1. Effects of bovine hypothalamic extract (BHE) on the proliferation of mouse epidermal melanocytes in primary culture[1]

Culture medium	Total number of melanocytes/ 35 mm dish (× 10^4)	
	2 days	12 days
F-10	0.08 ± 0.02	2.96 ± 1.28*[a]
F-10 + Ins	0.10 ± 0.04	2.88 ± 0.59*[b]
F-10 + Tf	0.07 ± 0.01	3.41 ± 0.88*[c]
F-10 + BHE	0.38 ± 0.10	1.83 ± 1.06*[d]
F-10 + BHE + Ins + Tf	0.67 ± 0.11	3.89 ± 0.55*[e]
F-10 + BHE + BPE + Ins + Tf	3.76 ± 0.42	25.24 ± 3.04*[f]
199 + dialyzed BHE + EGF + T_3 + Ins + Tf + HC + CT	0.61 ± 0.17	5.06 ± 0.84*[g]

[1] Suspensions of 2×10^6 epidermal cells were plated in 35 mm dishes and cultured with seven different media. FBS was added at a concentration of 4% for the first 2 days. BHE (100 μg/ml), BPE (10 μg/ml), Ins (10 μg/ml), Tf (10 μg/ml), dialyzed BHE (100 μg/ml), EGF (10 ng/ml), T_3 (10^{-9}M), HC (0.5 μg/ml), and CT (10^{-10}M) were added to the culture medium. The number of melanocytes was counted by phase-contrast and brightfield microscopy at 2 and 12 days after plating. Each value is the means ± SE (standard error) of results of triplicate experiments.

*[a-f] Significant difference by *t*-test: $P<0.05$. Nonsignificant differences: a–b, a–c, a–d, a–e, a–g.

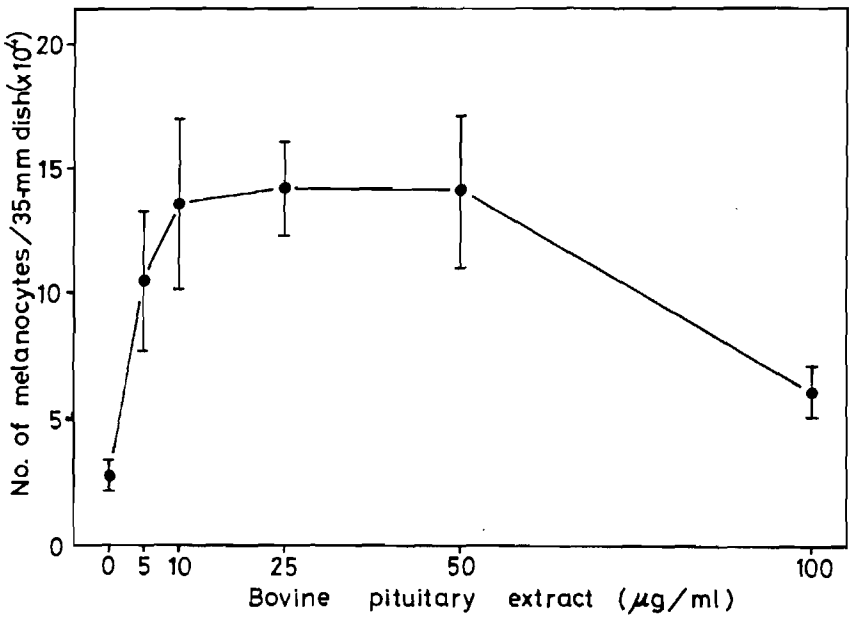

Fig. 3. Dose-response curve for mouse melanocytes grown for 12 days in a medium that consisted of F-10 plus BPE. FBS was added to a concentration of 4% for the first 2 days. The number of melanocytes was counted by phase-contrast and brightfield microscopy. Points plotted are the mean values of results from triplicate experiments with suspensions of epidermal cells from different litters of mice. Bars indicate the standard errors of the means.

Effects of various concentrations of BPE on the growth of melanocytes in primary culture

Suspensions of 2×10^6 epidermal cells were cultured with media that consisted of F-10 plus BPE at a concentration of 0, 5, 10, 25, 50, or 100 μg/ml. FBS was added at 4% to the culture medium for the first 2 days. After 12 days the number of melanocytes was counted directly under the phase-contrast and brightfield microscope. Melanocytes grew well over the range of concentrations of BPE from 10 to 50 μg/ml (Fig. 3).

Effects of various growth factors on the growth of melanocytes in primary culture

When the suspensions of epidermal cells were cultured with a medium that consisted of F-10 plus BHE (100 μg/ml) or F-10 plus BHE (100 μg/ml), Ins (10 μg/ml), and Tf (10 μg/ml), melanocytes became attached to the dishes but failed to grow and eventually died (Table 1). BHE was added to the culture media at a concentration of 10, 25, 50, and 150 μg/ml. BHE at these concentrations did not promote the growth of melanocytes (results not shown). Similarly, a complete medium used for growth of human epidermal melanocytes (Gibco Medium 199 plus 100 μg/ml of dialyzed BHE; 10 ng/ml of EGF; 10^{-9}M T_3; 10 μg/ml of Ins; 10 μg/ml of Tf; 0.5 μg/ml of HC; and 10^{-10}M CT; Hirobe et al., '88) did not induce the growth of mouse epidermal melanocytes (Table 1). Moreover, the combination of BHE and BPE brought about no additive increase in the rate of proliferation of melanocytes (Fig. 2B, Table 1).

EGF (10, 20, and 50 ng/ml), aFGF (0.5 and 5 ng/ml), bFGF (0.1, 1, and 10 ng/ml), NGF (5 ng/ml), T_3 (10^{-9}M), and HC (0.05, 0.5, 5, and 50 μg/ml) were tested for their growth-promoting activity in this culture system. None of these factors enhanced the growth of melanocytes (results not shown). Similarly, the combination of MGM with these factors brought about no additive increase in the rate of proliferation of melanocytes (results not shown).

Serial passage of mouse epidermal melanocytes

Melanocytes could be serially passaged in MGM supplemented with CM from the primary culture (passage medium) without contamination by keratinocytes and fibroblasts. At the time of plating, 1% FBS was added to a passage medium. Within 1 day after plating, melanocytes could be seen attached to the dish (Fig. 4A). At this stage, the polygonal melanocytes were outnumbered by bipolar, tripolar, and dendritic melanocytes (Fig. 4A). From the second day, FBS was excluded from the culture medium. After 2 days the dishes con-

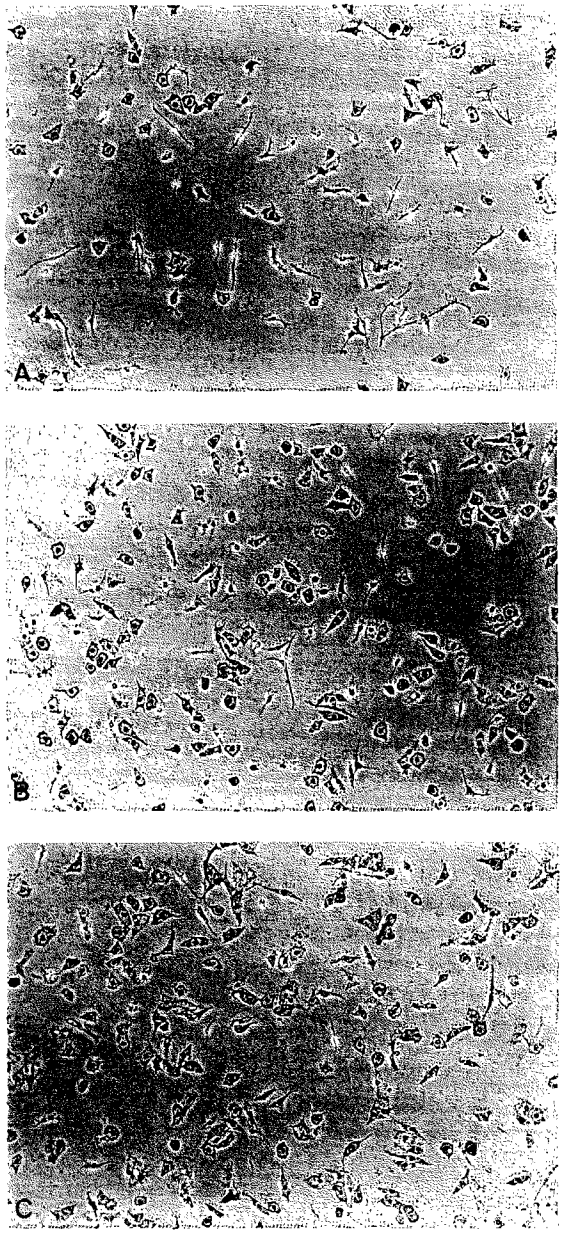

Fig. 4. Secondary cultures of mouse melanocytes in a passage medium that consisted of MGM plus conditioned medium (CM) from the primary culture. The CM was added to MGM at a ratio of 1:2 (v/v). CM from cultures 3 to 7 days after plating was used. The conditioning was continued for 1 day. FBS was added at a concentration of 1% for the first day. The plating density was 6×10^4 cells/35 mm dish. **A:** After 1 day in culture. Cultures consist entirely of bipolar to polygonal melanocytes. **B:** After 2 days in culture. The culture contains numerous melanocytes, and polygonal cells are more numerous than bipolar, tripolar, or dendritic cells. **C:** After 3 days in culture. The culture contains mostly polygonal or epithelioid melanocytes. Phase-contrast microscopy. ×60.

tained numerous melanocytes. Polygonal cells were more numerous than bipolar, tripolar, or dendritic cells at this stage (Fig. 4B). After 3 days the cultures contained mostly polygonal or epithelioid melanocytes (Fig. 4C). After 4 days the melanocytes gradually decreased in number. By contrast, serially passaged cultures of melanocytes grew neither in MGM without CM nor in BPE-free passage medium (Table 2).

The melanocytes in passage cultures seemed to be metabolically active, as almost all melanocytes synthesized melanin when supplied with exogenous dopa. Melanocytes in primary and passage cultures were viable and produced pigment granules except for a small number of melanocytes that died. Dead melanocytes could be distinguished morphologically from living melanocytes. The viability of melanocytes was also confirmed by trypan-blue exclusion test.

The growth-promoting activity of CM depends entirely on the stage of the primary culture at which it is collected. CM from the early stage of the primary cultures, which are enriched with keratinocytes (3, 5, and 7 days in culture), enhanced the proliferation of melanocytes, whereas CM from late-stage primary cultures, which consisted mainly of melanocytes (9, 12, and 14 days in culture), failed to support the proliferation of melanocytes (Table 2).

The kinetics of the proliferation of melanocytes in secondary cultures (first passage) were studied by direct counting of melanocytes in pure cultures under the microscope (Fig. 5A). A plating density of 6×10^4 cells/35 mm dish was found to be best because, at lower densities (2 and 4×10^4 cells/35 mm dish), melanocytes proliferated more slowly. With subsequent passaging, growth rates decreased (Figs. 5B, C), and melanocytes ceased to grow entirely by the fourth passage even though they remained viable and pigmented.

DISCUSSION

The culture system developed in this study permitted the selective growth of normal mouse melanocytes from disaggregated epidermal cells, a population of cells in which this type of cell consitutes only a minor fraction. BPE is specific in its ability to support the proliferation of melanocytes, as keratinocytes and fibroblasts are unable to proliferate in this medium. Ins and Tf are also essential for maintaining melanocytes in vitro. In serial passage, melanocytes failed to grow in the absence of CM. CM prepared from keratinocyte-enriched cultures enhanced the growth of melano-

TABLE 2. Effects of conditioned medium (CM) on the proliferation of mouse epidermal melanocytes in secondary culture[1]

Culture medium	Total number of melanocytes/ 35 mm dish ($\times 10^4$) 1 day	3 days
MGM	2.61 ± 0.45	1.70 ± 0.37*[a]
BPE alone	1.61 ± 0.63	1.15 ± 0.41*[b]
BPE-free MGM + BPE-free CM	1.92 ± 0.72	1.21 ± 0.35*[c]
Ins · Tf-free MGM + Ins · Tf-free CM	2.69 ± 1.03	2.61 ± 0.92*[d]
MGM + CM (3 days)	5.50 ± 1.95	9.69 ± 1.53*[e]
MGM + CM (5 days)	6.29 ± 1.44	12.81 ± 2.44*[f]
MGM + CM (7 days)	5.64 ± 1.56	9.35 ± 2.69*[g]
MGM + CM (9 days)	3.15 ± 0.62	3.72 ± 0.86*[h]
MGM + CM (12 days)	3.10 ± 0.70	2.30 ± 0.72*[i]
MGM + CM (14 days)	3.33 ± 0.97	2.24 ± 0.32*[j]

[1] Melanocytes in primary cultures were plated in 35 mm dishes at a density of 4×10^4 cells per dish and cultured with ten different media. FBS was added at a concentration of 1% for the first day. CM from cultures after 3 to 14 days of primary growth were used. The ratio of CM to MGM was 1:2 (v/v). The conditioning was continued for 1 day. The number of melanocytes was counted by phase-contrast and bright-field microscopy at 1 and 3 days after plating. Each value is the mean ± SE (standard error) of results of triplicate experiments.

*[a-g] Significant differences by *t*-test: a–e, a–f, a–g, $P<0.05$. Nonsignificant differences: a–b, a–c, a–d, a–h, a–i, a–j.

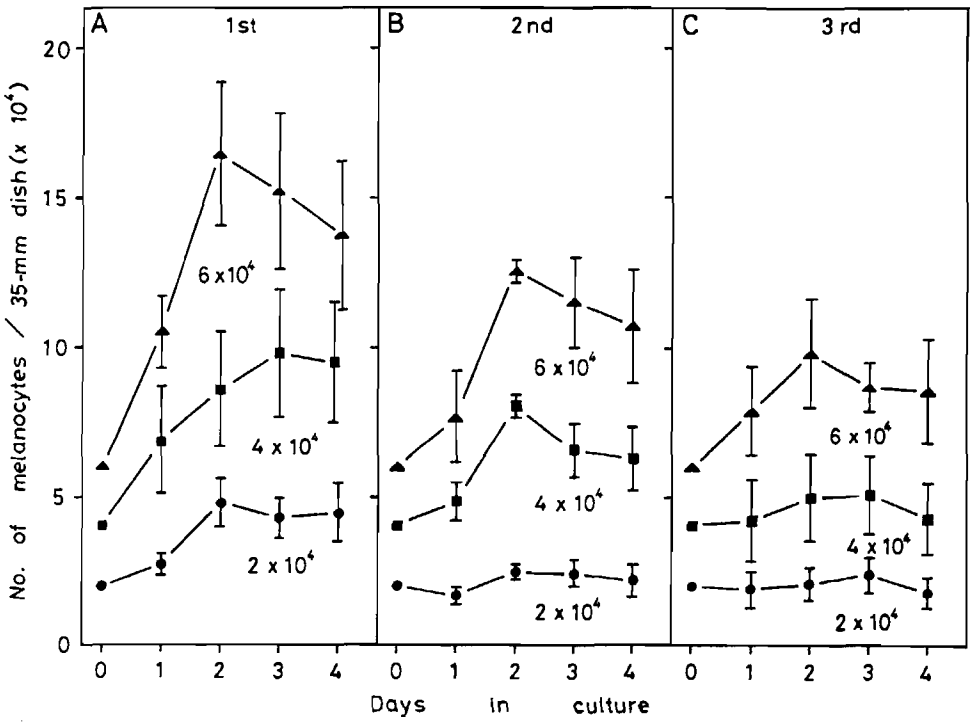

Fig. 5. Growth kinetics of mouse melanocytes. **A:** In secondary cultures (1st passage); **B:** Tertiary cultures (2nd passage); and **C:** Quaternary cultures (3rd passage). Melanocytes were plated in 35 mm dishes at densities of 2 (●), 4 (■), and 6 (▲) $\times 10^4$ cells per dish and cultured with a passage medium by the method described in the legend to Figure 4. The number of melanocytes was counted by phase-contrast and bright-field microscopy at 1, 2, 3, and 4 days after plating. The data are the averages of results of triplicate experiments with suspensions of melanocytes from different primary cultures or passage cultures. Bars indicate standard errors of the means.

cytes, whereas CM from melanocyte-enriched cultures did not. Therefore, it is probable that the mouse keratinocytes in primary culture release a factor into the culture medium that enhances the growth of melanocytes. It is also possible that melanocytes produce a factor that inhibits their own growth. However, these possibilities remain to be investigated in the future. Pure and enriched serially passaged cultures of mouse epidermal melanocytes can be used for examining the problems of the growth control of proliferation of mouse melanocytes (Quevedo and Smith, '63; Rosdahl and Szabo, '78; Hirobe, '83, '88a, b).

The important role of BPE in promoting the growth in culture of cells from normal mammalian tissues has been reported. BPE was first utilized in a system designed for the culture of normal human keratinocytes (Peehl and Ham, '80). BPE induced the growth of human keratinocytes without the requirements for a feeder layer or conditioned medium. BPE has been found to be essential for the culture of various kinds of epithelial cell, including human keratinocytes (Tsao et al., '82; Wille et al., '84), mouse keratinocytes (Bertolero et al., '84), human bronchus epithelial cells (Willey et al., '85), human mammary epithelial cells (Hammond et al., '84), and rat prostate epithelial cells (McKeehan et al., '84; Peehl et al., '88). Moreover, a dialyzed BPE was reported to stimulate the clonal growth of human muscle satellite cells (Ham et al., '88). Although a growth factor was purified from BPE that controlled the division of an ovarian cell line (Gospodarowicz et al., '74), most of the factors responsible for the growth-inducing activity have not yet been identified. Bertolero et al. ('84) reported that mouse keratinocytes could grow in Ca^{2+}-free minimum essential medium (MEM) supplemented with BPE and hormones. In the present study, mouse keratinocytes could not be maintained in a medium composed of F-10 plus BPE and hormones, a result that suggests that mouse keratinocytes cannot grow in Ca^{2+}-rich medium regardless of the presence of BPE.

BHE has been reported to support the proliferation in culture of human melanocytes in a hormone-supplemented medium without contamination by keratinocytes and fibroblasts (Wilkins et al., '82, '85; Gilchrest et al., '84; Hirobe et al., '88). However, the extract did not support the growth of mouse epidermal melanocytes in the present study. The results suggest that the growth factor required by human melanocytes and present in BHE is specific in its ability to support the growth of human epidermal melanocytes. However, it is not known at present whether or not the growth factor required by mouse melanocytes and present in BPE is specific for the induction of proliferation of mouse melanocytes. Purification and characterization of the growth factor required by mouse melanocytes remain to be pursued. It is of interest that the growth factor required by mammalian melanocytes is present in both BHE and BPE. Regulation of the proliferation of mammalian melanocytes by factors present in the hypothalamohypophysial system may reflect the neural crest origin of these cells (Rawles, '47).

De Luca et al. ('88) prepared pure human melanocytes from skin biopsies by culturing epidermal cells in a medium that contained TPA. They also prepared pure human keratinocytes from skin biopsies by culturing epidermal cells on a feeder layer of lethally irradiated 3T3 cells. These melanocytes and keratinocytes were cocultured in serially passaged cultures. De Luca et al., ('88) found that the increase in numbers of melanocytes that were cocultured with keratinocytes was more than tenfold greater than the increase in the numbers of melanocytes cultured without keratinocytes. From these experiments, they concluded that human keratinocytes possessed a factor(s) that stimulated the proliferation of human melanocytes. Gordon et al. ('89) reported that CM from human keratinocytes cultured in a hormonally defined medium for 1 day stimulated the growth of human epidermal melanocytes in the presence of BHE. Their results suggest that human keratinocytes release factors into the culture medium that enhance the growth of human epidermal melanocytes. The results of the present study provide support for the hypothesis that keratinocytes possess an activity that stimulates the growth of melanocytes.

Basic fibroblast growth factor (bFGF) was reported to enhance the growth in culture of human epidermal melanocytes derived from foreskins (Halaban et al., '87). Evidence was also presented that bFGF derived from keratinocytes was a natural source of mitogen for human epidermal melanocytes (Halaban et al., '88b). These results suggest that bFGF produced by human keratinocytes regulates the growth of human epidermal melanocytes. However, bFGF was unable to replace the growth-stimulating activity specific for mouse melanocytes found in BPE or CM in the present study. Although the differences between the results of Halaban et al. ('88b) and the present findings cannot be fully explained, they may be

attributable to differences in the type of tissue or in the species used.

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LITERATURE CITED

Abe, H., S. Aizu, and T. Takeuchi (1986) Establishment of a cultured melanocyte clone from albino mouse. In: Structure and Function of Melanin. K. Jimbow, ed. Fuji-shoin Co. Ltd., Sapporo, pp. 69–74.

Bennett, D.C., P.J. Cooper, and I.R. Hart (1987) A line of non-tumorigenic mouse melanocyte, syngeneic with B16 melanoma and requiring a tumor promoter for growth. Int. J. Cancer, *39*:414–418.

Bennett, D.C., P.J. Cooper, T.J. Dexter, L.M. Devlin, J. Heasman, and B. Nester (1989) Cloned mouse melanocyte lines carrying the germline mutations albino and brown: complementation in culture. Development, *105*:379–385.

Bertolero, F., M.E. Kaighn, M.A. Gonda, and U. Saffiotti (1984) Mouse epidermal keratinocytes. Clonal proliferation and response to hormones and growth factors in serum-free medium. Exp. Cell Res., *155*:64–80.

De Luca, M., F. D'Anna, S. Bondanza, A. Tito Franzi, and R. Cancedda (1988) Human epithelial cells induce human melancoyte growth in vitro but only skin keratinocytes regulate its proper differentiation in the absence of dermis. J. Cell Biol., *107*:1919–1926.

Eisinger, M., and O. Marko (1982) Selective proliferation of normal human melanocytes in vitro in the presence of phorbol ester and cholera toxin. Proc. Natl. Acad. Sci. U.S.A., *79*:2018–2022.

Gerson, D.E., and G. Szabó (1968) Effect of single gene substitution on the melanocyte system of the C57Bl mouse: Quantitative and qualitative histology. Nature, *218*:381–382.

Gilchrest, B.A., M.A. Vrabel, E. Flynn, and G. Szabo (1984) Selective cultivation of human melanocytes from newborn and adult epidermis. J. Invest. Dermatol., *83*:370–376.

Gordon, P.R., C.P. Mansur, and B.A. Gilchrest (1989) Regulation of human melanocyte growth, dendricity, and melanization by keratinocyte-derived factors. J. Invest. Dermatol., *92*:565–572.

Gospodarowicz, D., K.L. Jones, and G. Sato (1974) Purification of a growth factor for ovarian cells from bovine pituitary glands. Proc. Natl. Acad. Sci. U.S.A., *71*:2295–2299.

Halaban, R., S. Ghosh, and A. Baird (1987) bFGF is a putative natural growth factor for human melanocytes. In Vitro Cell. Dev. Biol., *23*:47–52.

Halaban, R., G. Moellman, A. Tamura, B.S. Kwon, E. Kuklinska, S.H. Pomerantz, and A.B. Lerner (1988a) Tyrosinases of murine melanocytes with mutations at the albino locus. Proc. Natl. Acad. Sci. U.S.A., *85*:7241–7245.

Halaban, R., R. Langdon, N. Birchall, C. Cuono, A. Baird, G. Scott, G. Moellmann, and J. McGuire (1988b) Basic fibroblast growth factor from human keratinocytes is a natural mitogen for melanocytes. J. Cell Biol., *107*:1611–1619.

Ham, R.G., J.A. St. Clair, C. Webster, and H.M. Blau (1988) Improved media for normal human muscle satellite cells: Serum-free clonal growth and enhanced growth with low serum. In Vitro Cell. Dev. Biol., *24*:833–844.

Hammond, S.L., R.G. Ham, and M.R. Stampfer (1984) Serum-free growth of human mammary epithelial cells: Rapid clonal growth in defined medium and extended serial passage with pituitary extract. Proc. Natl. Acad. Sci. U.S.A., *81*:5435–5439.

Hirobe, T. (1982) Genes involved in regulating the melanocyte and melanoblast-melanocyte populations in the epidermis of newborn mouse skin. J. Exp. Zool., *223*:257–264.

Hirobe, T. (1983) Proliferation of epidermal melanocytes during the healing of skin wounds in newborn mice. J. Exp. Zool., *227*:423–432.

Hirobe, T. (1984a) Histochemical survey of the distribution of the epidermal melanoblasts and melanocytes in the mouse during fetal and postnatal periods. Anat. Rec., *208*:589–594.

Hirobe, T. (1984b) Effects of genic substitution at the brown locus on the differentiation of epidermal melanocytes in newborn mouse skin. Anat. Rec., *209*:425–432.

Hirobe, T. (1987) Genetic control of the population size of the melanocyte in the mouse epidermis. Jpn. J. Genet., *62*:149–158.

Hirobe, T. (1988a) Developmental changes of the proliferative response of mouse epidermal melanocytes to skin wounding. Development, *102*:567–574.

Hirobe, T. (1988b) Genetic factors controlling the proliferative activity of mouse epidermal melanocytes during the healing of skin wounds. Genetics, *120*:551–558.

Hirobe, T., and T. Takeuchi (1977) Induction of melanogenesis in the epidermal melanoblasts of newborn mouse skin by MSH. J. Embryol. Exp. Morphol., *37*:79–90.

Hirobe, T., and T. Takeuchi (1978) Changes of organelles associated with the differentiation of epidermal melanocytes in the mouse. J. Embryol. Exp. Morphol., *43*:107–121.

Hirobe, T., E. Flynn, G. Szabo, M. Vrabel, and R.I. Garcia (1988) Growth characteristics of human epidermal melanocytes in pure culture with special reference to genetic differences. J. Cell. Physiol., *135*:262–268.

Ito, K., and T. Takeuchi (1981) Melanocyte differentiation in black-eyed white mice. In: Pigment Cell 1981, Phenotypic Expression in Pigment Cells. M. Seiji, ed. Univ. Tokyo Press, Tokyo, p. 185–188.

Koyama, Y., and T. Takeuchi (1981) Ultrastructural observations on melanosome aggregation in genetically defective melanocytes of the mouse. Anat. Rec., *201*:599–611.

Mayer, T.C. (1973) The migratory pathway of neural crest cells into the skin of mouse embryos. Dev. Biol., *34*:39–46.

Mayer, T.C. (1982) The control of embryonic pigment cell proliferation in culture by cyclic AMP. Dev. Biol., *94*:509–514.

Mayer, T.C., and L. Oddis (1977) Pigment cell differentiation in embryonic mouse skin and isolated epidermis: an in vitro study. J. Exp. Zool., *202*:415–424.

McKeehan, W.L., P.S. Adams, and M.P. Kosser (1984) Direct mitogenic effects of insulin, epidermal growth factor, glucocorticoid, cholera toxin, unknown pituitary factors and possibly prolactin, but not androgen, on normal rat prostate epithelial cells in serum-free, primary cell culture. Cancer Res., *44*:1998–2010.

Peehl, D.M., and R.G. Ham (1980) Growth and differentiation of human keratinocytes without a feeder layer or conditioned medium. In Vitro, *16*:526–538.

Peehl, D.M., S.T. Wong, and T.A. Stamey (1988) Clonal

growth characteristics of adult human prostatic epithelial cells. In Vitro Cell. Dev. Biol., *24:*530–536.

Quevedo, W.C., Jr., and J.A. Smith (1963) Studies on radiation-induced tanning of skin. Ann. N.Y. Acad. Sci., *100:* 364–388.

Quevedo, W.C., Jr., M.C. Youle, D.T. Rovee, and T.C. Bienieki (1966) The developmental fate of melanocytes in murine skin. In: Structure and Control of the Melanocyte. G. Della Porta, and O. Mühlbock, eds. Springer-Verlag, Berlin, pp. 228–241.

Rawles, M.E. (1947) Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zool., *20:*248–266.

Rosdahl, I.K. (1978) Melanocyte mitosis in UVB-irradiated mouse skin. Acta Derm. Venereol. (Stockh.), *58:*217–221.

Rosdahl, I.K., and G. Szabo (1978) Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. J. Invest. Dermatol., *70:*143–148.

Sato, C., S. Ito, and T. Takeuchi (1985) Establishment of a mouse melanocyte clone within synthesizes both eumelanin and pheomelanin. Cell Struct. Funct., *10:*421–425.

Takeuchi, T. (1968) Genetic analysis of a factor regulating melanogenesis in the mouse melanocytes. Jpn. J. Genet., *43:*249–256.

Tamate, H.B., T. Hirobe, and T. Takeuchi (1986) Effects of lethal yellow (A^y) and recessive yellow (*e*) genes on the population of epidermal melanocytes in newborn mice. J. Exp. Zool., *238:*235–240.

Tamura, A., R. Halaban, G. Moellman, J.M. Cowan, M.R. Lerner, and A.B. Lerner (1987) Normal murine melanocytes in culture. In Vitro Cell. Dev. Biol., *23:*519–522.

Tsao, M.C., B.J. Walthall, and R.G. Ham (1982) Clonal growth of normal human epidermal keratinocytes in a defined medium. J. Cell. Physiol., *110:*219–229.

Weiss, L.M., and A.S. Zelickson (1975) Embryology of the epidermis: ultrastructural aspects. III. Maturation and primary appearance of dendritic cells in the mouse with mammalian comparisons. Acta Derm. Venereol. (Stockh.), *55:* 431–442.

Wilkins, L.M., B.A. Gilchrest, T. Maciag, G. Szabo, and L. Connell (1982) Growth of enriched human melanocyte cultures. In: Growth of Cells in Hormonally Defined Media. G. Sato, A. Pardee, and D. Sirbasku, eds. Cold Spring Harbor Press, New York, pp. 929–936.

Wilkins, L.M., B.A. Gilchrest, G. Szabo, R. Weinstein, and T. Maciag (1985) The stimulation of normal human melanocyte proliferation in vitro by melanocyte growth factor from bovine brain. J. Cell. Physiol., *122:*350–361.

Wille, J.J., Jr., M.R. Pittelkow, G.D. Shipley, and R.E. Scott (1984) Integrated control of growth and differentiation of normal human prokeratinocytes cultured in serum-free medium: clonal analyses, growth kinetics, and cell cycle studies. J. Cell. Physiol., *121:*31–44.

Willey, J.C., M.A. Laveck, I.A. McCledon, and J.F. Lechner (1985) Relationship of ornithine decarboxylase activity and cAMP metabolism to proliferation of normal human bronchial epithelial cells. J. Cell. Physiol., *124:*207–212.

Exhibit C

PATENT
Attorney Docket No.: AVT-001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Wood et al.	CONF. NO:	8540
APPLICATION NO.:	10/068,299	GROUP NO:	1651
FILING DATE:	February 6, 2002	EXAMINER:	Barnhart, Lora Elizabeth
TITLE:	CELL SUSPENSION PREPARATION TECHNIQUE AND DEVICE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT AND RESPONSE

Sir:

Applicants submit this Amendment and Response in response to the Office action mailed from the United States Patent and Trademark Office on December 30, 2009. Applicants submit herewith a petition for one-month extension of time. An affidavit by Dr. Fiona M. Wood under 37 C.F.R. § 1.132 is also submitted herewith. The Commissioner is authorized to charge any fees occasioned by entry of this paper to Attorney's Deposit Account No. 50-3081.

Applicants respectfully request entry of this Amendment and Remarks, in which:

- **Amendments to the Claims** begin on page 2.
- **Remarks** begin on page 9.

Amendments to the Claims

This listing of the claims will replace all prior versions and listings of the claims in the application.

Listing of Claims

1-28. (Canceled)

29. (Currently amended) A cell suspension produced according to a method comprising the steps of:

(a) physically and/or chemically dissociating cellular stratum in ~~a dermal-epithelial~~ skin tissue sample obtained from a patient to provide cells suitable for grafting to the patient, wherein the skin tissue sample comprises dermis, epidermis, and a dermal-epidermal junction therebetween;

(b) harvesting [[the]] cells from the dermis and the epidermis in the presence of a nutrient solution, the harvested cells having the potential to include cellular congregates; and

(c) filtering the cells in nutrient solution to remove cellular congregates greater than 200 μM,

wherein the resulting cell suspension is free of serum xenogenic to said patient and of cellular congregates greater than 200 μM, and

wherein the resulting cell suspension comprises a composition of viable cells autologous to said patient, and wherein said composition has ~~a ratio of~~ a cell population comprising keratinocyte basal cells, melanocytes and fibroblasts ~~that is~~, the cell population of the composition and the skin tissue sample being comparable ~~to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in said tissue sample~~.

30-33. (Canceled)

34. (Withdrawn) A cell suspension according to claim 29, the suspension being distributed on a patient tissue site undergoing tissue grafting.

35. (Withdrawn) A cell suspension according to claim 29 comprising the further step of:

(d) administering the suspension directly to a region on the patient that requires a cell graft.

36. (Withdrawn) A cell suspension according to claim 35 wherein the tissue sample is obtained from the patient that requires a cell graft.

37. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is distributed relatively evenly over the graft region.

38. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively.

39. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension contains cells present in a ratio to each other comparable to those in the donor sample.

40. (Withdrawn) A cell suspension according to claim 39 wherein the cells include keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

41. (Withdrawn) A suspension according to claim 40 wherein the cells are substantially viable.

42. (Withdrawn) A cell suspension according to claim 37 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

43. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively from a tissue sample for the patient that requires a cell graft, contains cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

44. (Withdrawn) A cell suspension produced according to a method comprising the steps of:

(a) obtaining a tissue sample from a site on a donor in need of a tissue graft;

(b) physically and/or chemically dissociating and removing cellular stratum from cells present in the sample;

(c) harvesting the cells in the presence of a nutrient solution;

(d) distributing the suspension on a site of the donor as an autologous tissue graft.

45. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of cell conglomerates.

46. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of xenogenic serum.

47. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is distributed relatively evenly over the graft region.

48. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is obtained perioperatively.

49. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension contains cells represent in a ratio to each other comparable to those seen in the donor sample.

50. (Withdrawn) A suspension according to claim 49 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

51. (Withdrawn) A suspension according to claim 50 wherein the cells are substantially viable.

52. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is sprayed, spread, pipetted, or pained on to the tissue site.

53. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is obtained perioperatively, contains cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

54. (Withdrawn) A cell suspension produced by a method comprising obtaining cells from a patient in need of a tissue graft, providing the cells in nutrient solution in a manner that is substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension being distributed in apposition to the site of the recipient as a tissue graft.

55. (Withdrawn) A suspension according to claim 54 wherein the suspension is distributed relatively evenly over the graft region.

56. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension is obtained perioperatively.

57. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

58. (Withdrawn) A suspension according to claim 57 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

59. (Withdrawn) A suspension according to claim 58 wherein the cells are substantially viable.

60. (Withdrawn) A cell suspension according to claim 55 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

61. (Currently amended) A cell suspension comprising cells ~~derived~~ harvested from a ~~dermal-epithelial~~ skin tissue sample obtained from a patient, wherein the skin tissue sample comprises

dermis, epidermis, and a dermal-epidermal junction therebetween, the cell suspension comprising:

(a) a composition of viable cells ~~derived~~ harvested from the dermis and the epidermis of said skin tissue sample and autologous to said patient, said composition having ~~a ratio of~~ a cell population comprising keratinocyte basal cells, fibroblasts and melanocytes, the cell population of the composition and the skin tissue sample being comparable ~~that is comparable to a ratio of keratinocyte basal cells, fibroblasts and melanocytes present in said tissue sample~~; and

(b) a nutrient solution free of serum xenogenic to the patient,

wherein said cell suspension is free of cellular congregates greater than 200 μm.

62. (Withdrawn) A suspension according to claim 61 wherein the suspension is distributed relatively evenly over the graft region.

63. (Previously presented) A cell suspension according to claim 61 wherein the cell suspension is obtained perioperatively.

64. (Canceled)

65. (Currently amended) A suspension according to claim 61 wherein said composition of cells ~~comprise~~ comprises keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

66. (Canceled)

67. (Withdrawn) A cell suspension according to claim 62, wherein the cell suspension is sprayed, spread pipetted, or painted onto the tissue site.

68. (Withdrawn) A cell suspension produced by a method sufficient to provide the cells in nutrient solution, substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension serving as a graft in apposition to the body of a recipient in need of a tissue graft.

69. (Withdrawn) A suspension according to claim 68 wherein the suspension is distributed relatively evenly over the graft region.

70. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is obtained perioperatively.

71. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

72. (Withdrawn) A suspension according to claim 71 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

73. (Withdrawn) A suspension according to claim 72 wherein the cells are substantially viable.

74. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

75. (Currently amended) A cell suspension according to claim 61, ~~further~~ wherein the viable cells are harvested in a method using a solution comprising an enzyme.

76. (Currently amended) A cell suspension according to claim 75 wherein the enzyme is selected from the group consisting of trypsin, ~~trypsin-edta~~ trypsin-EDTA, dispase, collagenase, thermolysin, pronase, hyaluronidase, pancreatin, elastase, and papain.

77. (Previously presented) A cell suspension according to claim 76 wherein the enzyme is trypsin.

78. (Currently amended) A cell suspension according to claim 77 wherein the trypsin is present in [[a]] the solution in an amount that is between 5 and 0.1% per volume of the solution.

79. (Currently amended) A cell suspension according to claim 77 wherein the trypsin is present in [[a]] the solution in an amount that is less than 0.05% per volume of the solution.

REMARKS

Claims

Claims 29, 61, 63, 65, and 75-79 were considered in the non-final Office Action of December 30, 2009. Claims 34-60, 62, and 67-74 stand withdrawn from consideration. Claims 65 and 76 stand objected to and claims 29, 61, 63, 65, and 75-79 stand rejected.

Claims 29 and 61 are amended herein to clarify that the tissue sample is a skin tissue sample comprising dermis, epidermis, and a dermal-epidermal junction therebetween. Claims 29 and 61 have also been amended to provide that the cells are harvested from the dermis and the epidermis. These amendments are supported in the specification as filed at least at page 11, lines 9-11 and page 22, lines 10-14. Claims 29 and 61 have also been amended to recite that the composition has a cell population comprising keratinocyte basal cells, melanocytes and fibroblasts. This amendment is supported in the specification as filed at least at page 8, lines 18-21; and at page 12, lines 26-30. Further, claims 29 and 61 have been amended to recite that the composition and the tissue sample have cell compositions that are comparable. Claim 29 has also been amended to recite the "cell suspension" where appropriate to provide proper antecedent basis.

Claim 75, from which claims 78 and 79 indirectly depend, has been amended to clarify that the cells are harvested in a method using a solution that comprises an enzyme. This amendment is supported in the specification as filed at least at page 9, lines 16-22. In view of the amendment to claim 75, claims 78 and 79 have been amended to provide proper antecedent basis.

Further, as suggested by the Examiner, claim 65 has been amended to recite "comprises", and claim 76 is amended to recite "trypsin-EDTA" to correct for clerical errors.

No new matter has been added herein. Applicants have amended certain claims solely to expedite prosecution of the application. In making these amendments, Applicants are not acquiescing to the pending rejections and are not abandoning or surrendering any of the subject matter in previous versions or listings of the claims or in the application. Accordingly, Applicants reserve the right to pursue claims of similar, narrower, or broader scope in the future.

In view of the amendments to the claims and the following remarks, together with the affidavit under 37 C.F.R. § 1.132 by Dr. Fiona M. Wood (submitted herewith), Applicants respectfully request reconsideration and withdrawal of all claim objections and rejections.

Summary of Interview

As an initial matter, Applicants wish to thank the Examiner for the telephonic interview of March 23, 2010, during which the Examiner, the undersigned attorney, and Dr. William Dolphin were present. The presently-claimed invention and the following prior art references were discussed at the Interview: Noel-Hudson et al. (1993, *In Vitro Cell and Developmental Biology – Animal* 31: 508-515, reference C6 on 6/1/04 IDS, "Noel-Hudson"); and Hirobe (1991, *Journal of Experimental Zoology* 257: 184-194; reference U, "Hirobe 1991"). In discussing Noel-Hudson, Applicants noted that Noel-Hudson describes certain methods, in part, by reference to Boyce, S.T., Ham, R.G., "Cultivation, frozen storage and clonal growth of normal human epithelial keratinocytes in serum-free media." *J. Tissue Cult. Methods* 9:83-93 (1985) ("Boyce and Ham")[1], and Applicants further discussed Boyce and Ham. In addition, during the Interview, the rejections made under 35 USC 112 were discussed, and Applicants described certain amendments reflected herein. As required under MPEP § 713.04, Applicants respectfully supplement the Examiner Interview Summary mailed March 25, 2010 with the following additional information:

(A) No exhibit was shown and no demonstration was conducted during the Interview;

(B) Certain limitations present in independent claims 29 and 61 were discussed during the Interview;

(C) Noel-Hudson, Hirobe, and Boyce and Ham were discussed during the Interview;

(D) The amendments made herein were discussed by Applicants during the Interview;

(E) The general thrust of the principal argument of the Applicants was that the references fail to disclose the elements of the invention as claimed, including a composition of cells harvested from a tissue sample comprising dermis, epidermis and a dermal-epidermal junction therebetween; the composition being free of xenogenic serum and cellular congregates greater that 200 μM, and having a cell population comprising

[1] A copy of Boyce and Ham is submitted herewith in an Information Disclosure Statement.

keratinocyte basal cells, melanocytes and fibroblasts, wherein the cell population of the composition and the tissue sample are comparable.

(F) No other pertinent matters were discussed during the Interview; and

(G) The Interview did not result in any agreement between the Applicants and the Examiner.

Objection to Claims 65 and 76

Claims 65 and 76 were objected to for informalities. As discussed above, Applicants have amended claims 65 and 76 according to the Examiner's suggestion. Accordingly, Applicants respectfully request reconsideration and withdrawal of the objections to claims 65 and 76.

Rejection Under 35 U.S.C. § 112, Second Paragraph

In the Office action, claims 29, 61, 63, 65, and 75-79 were rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter regarded by the Applicants as the invention. Specifically, the Examiner requested clarification of "dermal-epithelial" and "a ratio…comparable" in claims 29 and 61, "derived" in claim 61, and trypsin concentration in claims 78 and 79.

Regarding the recitation of "dermal-epithelial", Applicants have amended claims 29 and 61 to delete the reference to the dermal-epithelial junction and to recite that the tissue sample is a skin tissue sample comprising dermis, epidermis, and a dermal-epidermal junction therebetween. Claims 29 and 61 have also been amended to recite that the cell composition is produced by harvesting cells from both the dermis and epidermis, and that the cell composition thus produced comprises keratinocyte basal cells, melanocytes, and fibroblasts. As amended, Applicants respectfully submit that claims 29 and 61 are not indefinite with respect to the recitation of the sample or the location from which the cells are obtained.

Regarding the recitation of "a ratio…comparable", the Examiner stated that the use of the term "ratio" in connection with three value was confusing. In addition, the Examiner indicated that the criteria for determining whether one ratio is comparable to another are not clear. In response, Applicants have amended claims 29 and 61 to delete the reference to a ratio and to

recite that the cell population of the composition (which comprises keratinocyte basal cells, melanoctyes, and fibroblasts) and tissue sample are comparable—i.e., similar. With regard to "comparable," Applicants respectfully submit that this expression is clear and definite in the context of the claimed method of producing a cell suspension from a tissue sample. As provided in the specification at page 12, lines 23-30:

> "Another unique feature of the cell suspension produced according to the method of the first aspect of the invention is that the composition of cells in the cellular preparation is comparable to that seen in situ compared to prior art cellular preparation. One possible explanation for this is that in the prior art, culture of the cellular preparation utilises selective culture for keratinocytes, therefore loss of cellular constituents such as fibroblasts and melanocytes occurs whereas the cellular suspension produced from the first aspect of the invention has a cell composition comparable to the in situ cell population."

In addition, Applicants explain in the specification at page 8, lines 18-24 that:

> "It provides a means to produce a suspension of cells in a ratio to each other comparable with those seen in situ. That is, due to the manner of preparation of the cellular suspension, cells such as keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes typically have enhanced survival rates in comparison to standard tissue culture techniques, whereby selective cell culture can result in the loss of certain cell types."

As such, it would be clear to a person of ordinary skills in the art that Applicants' methods (e.g., obtaining cells from the both dermis and epidermis of a skin tissue sample; use of a nutrient solution as opposed to a selective culture medium; and no separating or filtering of any particular cell type or tissue type) would produce a composition having a population of cell types that is comparable—i.e., similar—to the cell population of the tissue sample from which the composition is produced.

In addition to the foregoing, Applicants respectfully submit herewith an affidavit under 37 C.F.R. § 1.132 by Dr. Fiona M. Wood, which further confirms that Applicants' "cell population of the composition and the tissue sample are comparable (i.e., similar)." *Id.* at paragraph 5A. Therefore, in view of the above clarification and the affidavit, Applicants respectfully submit that the "comparable" expression is clear and definite.

Claim 61 has also been amended to clarify that the cell suspension comprises cells "harvested" from both the dermis and the epidermis. Applicants believe that this amendment address the Examiner's question regarding "derived."

Claim 75, from which claims 78 and 79 indirectly depend, has been amended to clarify that the cells are harvested in a method using a solution that comprises an enzyme. Applicants believe that these amendments clarify that the recited concentration of trypsin relates to the solution used in the harvesting method of the cells, as the Examiner had suggested. As such, Applicants believe these amendments fully address the Examiner's clarity rejection to claims 78 and 79.

In view of the foregoing amendments and clarification, together with the affidavit under 37 C.F.R. § 1.132 by Dr. Wood, Applicants respectfully request withdrawal and reconsideration of the rejections under 35 U.S.C. §112, second paragraph.

Rejection Under 35 U.S.C. § 102(b) Over Noel-Hudson

Independent claims 29 and 61 and dependent claim 63, 65, and 75-79 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Noel-Hudson et al. (1993, *In Vitro Cell and Developmental Biology – Animal* 31: 508-515, reference C6 on 6/1/04 IDS). In order for a claim to be anticipated, each and every element of the claim must be present in a single prior art reference. Applicants respectfully submit that Noel-Hudson does not anticipate each of claims 29, 61, 63, 65, and 75-79, at least because Noel-Hudson does not disclose each and every element of independent claims 29 and 61, from which claims 63, 65, and 75-79 directly or indirectly depend.

Independent claims 29 and 61 recite a cell suspension comprising a composition of cells harvested from the dermis and epidermis of a skin tissue sample. The composition of cells has a cell population comprising keratinocytes basal cells, melanocytes, and fibroblasts; and the cell population of the composition and the tissue sample are comparable. Claims 29 and 61 also recite that the cell suspension is free of cellular congregates greater than 200 μM.

Noel-Hudson reports the expression and differentiation of keratinocytes cultured on cell culture inserts. The keratinocytes were isolated from human foreskin according to the methods

of Boyce, S.T.; Ham, R.G., "Cultivation, frozen storage and clonal growth of normal human epithelial keratinocytes in serum-free media." *J. Tissue Cult. Methods* 9:83-93 (1985) ("Boyce and Ham"), as reported in Noel-Hudson at page 509, column 1, paragraph 7:

> "*Cells and culture conditions*. Human keratinocytes were isolated from human foreskin of 1-yr-old donor, as described by Boyce and Ham (7). Briefly, the biopsy fragments were first treated with 0.25% trypsin (wt/vol) and 1000 U/ml collagenase (vol/vol) in Hank's solution containing Ca^{++} for 2 h at 37° C, then with a 0.025% trypsin (wt/vol): 0.01% EDTA (wt/vol) solution to release individual cells."

Drawing from this paragraph, the Office action states at pages 5-6 that "Noel-Hudson's composition lacks all cell aggregates of any size ('individual cells;" ibid.) and comprises a physiological saline, specifically Hanks' solution with calcium salts (ibid.)." The Office action points to Van Bossuyt (U.S. Pat. No. 5,866,167, "Van Bossuyt") for support that "skin (such as the skin biopsy fragments of Noel-Hudson) inherently contains keratinocytes (epithelial cells), melanocytes and Langerhans cells in the epidermis; proliferating keratinocytes at the base of the epidermis; and fibroblasts in the dermis." The Office action further states that "Van Bossuyt's teachings indicate that the whole-skin biopsy of Noel-Hudson inherently contains all of the cell types recited in claims 29, 61 and 65." From this, the Office action concludes that "[t]he cited art taken as a whole demonstrates a reasonable probability that the suspension of the prior art is either identical or sufficiently similar to the claimed suspension that whatever differences exist are not patentably significant." According to the Office action, "[c]lear evidence that the suspension of the cited prior art does not possess a critical characteristic that is possessed by the claimed suspension (e.g., the presence of all of the recited cell types) would advance prosecution and might permit allowance of claims to applicant's suspension."

In response, Applicants respectfully submit that the cell suspension reported at page 509, column 1, paragraph 7 of Noel-Hudson is neither identical nor similar to the cell suspension claimed in the instant invention. Noel-Hudson states that the keratinocytes were isolated using the method described by Boyce and Ham. Turning to Boyce and Ham, each step used in the isolation and primary culture of human keratinocytes is described starting at page 85, Section H. In brief, at step 5, Boyce and Ham describe exposing 4-mm fragments of human foreskin to

collagenase under certain conditions until the "epidermis is readily removable from dermis." (Emphasis added.) At step 7, Boyce and Ham provide "[f]or each piece of tissue, remove the epidermis from the dermis by holding the dermis with fine mouse-tooth forceps and pulling the epidermis from the edge with needle-tipped forceps. The epidermis should separate as an intact sheet." (Emphasis added.) At step 8, Boyce and Ham provide "[a]s each piece of tissue is separated, place the epidermal layer in a 60-mm petri dish containing Solution A at room temperature. The dermis is discarded, unless it is used to culture dermal cell types."[2] (Emphasis added.) Boyce and Ham go on at step 9 to provide that the epidermal sheets are treated as follows: "[A]dd 6 ml of a solution of 0.025% trypsin (wt/vol) and 0.01% EDTA (wt/vol) in Solution A (pH 7.4). Gently agitate the epidermal fragments up and down in a cotton-plugged, sterile Pasteur pipette for 3 to 4 min to release individual cells…Using a sterile Pasteur pipette, withdraw the cell suspension from the remaining tissue fragments…and centrifuge..." (Emphasis added.) At step 10, Boyce and Ham provide that the pellet from centrifugation is resuspended "by gentle pipetting until it is broken into ***many small clumps***. Release more individual cells form the epidermal pieces in the petri dish by repeating the agitation…Transfer the resulting cell suspension (but not the remaining tissue fragments) from the dish…" (Emphasis added.) Boyce and Ham next describe centrifuging the cell suspension, and then at step 11 provide as follows: "[a]spirate the trypsin-inhibitor or serum solution from the pellet, resuspend the pellet in 2 to 3 ml of supplemented stock culture medium and gently pipette until the pellet is dispersed into a suspension of single cells ***(with some small clumps remaining)***. Count the cells…and inoculate the cells into pre-equilibrated flasks…" (Emphasis added). Boyce and Ham then provide for culturing, subculturing, and freezing. No further filtering or cell separation is described in Boyce and Ham.

Taken together with Boyce and Ham, Noel-Hudson reports at page 509, column 1, paragraph 7, a cell suspension comprising individual cells, along with small clumps, derived from the epidermis of a whole skin tissue sample. Nothing in Noel-Hudson teaches or suggests a

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[2] Boyce and Ham show in FIG. 1 that the dermis and epidermis are separated from each other and that only epidermis is subject to further treatment to release individual cells. Further, Applicants respectfully note that Noel-Hudson discloses the isolation of keratinocytes from the epidermis, and does not the use of the dermis to culture for dermal cells. Indeed, Noel-Hudson discloses "[c]ell suspensions of keratinocytes … in the absence of fibroblasts." Noel-Hudson at Abstract, emphasis added.

cell suspension free of cellular congregates greater than 200 μM. To the contrary, the cell suspension of Noel-Hudson includes small clumps—the brief description of isolating keratinocytes and the reference to releasing individual cells in Noel-Hudson corresponds to step 9 of Boyce and Ham which, at steps explicitly describes small clumps of cells remaining in the cell suspension. Furthermore, Noel-Hudson fails to teach or suggest, inherently or otherwise, a cell suspension that has a cell composition having a cell population comprising keratinocyte basal cells, melanocytes, ***and fibroblasts***—i.e., a composition of cells harvested from both the epidermis (e.g., keratinocyte basal cells and melanocytes) ***and the dermis*** (e.g., fibroblasts)[3]. Moreover, Noel-Hudson completely fails to teach or suggest a suspension having a cell composition that is comparable in cell population with the tissue sample from which the composition was produced—a tissue sample comprising both dermis and epidermis. In fact, because Noel-Hudson teaches separating the epidermal layer from the dermal layer and using only the epidermal layer of the skin tissue sample to produce the cell suspension, Noel-Hudson necessarily teaches away from the cell suspension of claims 29 and 61.

In addition to the foregoing, the affidavit under 37 C.F.R. § 1.132 by Dr. Wood further emphasizes that Noel-Hudson's composition is neither identical nor substantially similar to Applicants' cell suspension. See, for example, paragraph 5B where Dr. Wood states that: "Boyce removes the epidermis from the dermis, discards the dermis, uses a Pasteur pipette to agitate the epidermal fragments, and releases keratinocytes from the epidermal pieces. Boyce at pages 85-88. At least because the epidermis is separated from the dermis before any cell suspension is prepared, and because the cell suspension prepared by gentle pipetting contains many small clumps (thus not free of cellular congregates greater than 200 μM), Noel-Hudson's composition (in view of Boyce) is different from U.S.S.N. 10/068,299. That is, at no time does Noel-Hudson produce a composition that is either identical or substantially similar to the cell suspension of U.S.S.N. 10/068,299."

For at least the foregoing reasons, Applicants submit that claims 29 and 61 are patentable over Noel-Hudson. Claims 63, 65, and 75-79 are dependent upon claim 61, and thus are also patentable over Noel-Hudson. Accordingly, Applicants respectfully request that the rejection of

[3] Applicants respectfully refer the Examiner to Van Bossuyt at column 1, lines 40-50, which reports that keratinocytes and melanocytes are located in the epidermis, while fibroblasts are located in the dermis.

claims 29, 61, 63, 65, and 75-79 under 35 U.S.C. § 102(b) over Noel-Hudson be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 102(b) Over Hirobe

Independent claims 29 and 61 and dependent claim 63, 65, and 75-79 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hirobe (1991, *Journal of Experimental Zoology* 257: 184-194; reference U; "Hirobe 1991"). In order for a claim to be anticipated each and every element of the claim must be present in the cited art. Applicants respectfully submit that Hirobe 1991 does not anticipate each of claims 29, 61, 63, 65, and 75-79, at least because Hirobe does not disclose each and every element of claims 29 and 61, from which claims 63, 65, and 75-79 directly or indirectly depend.

Independent claims 29 and 61 recite a cell suspension comprising a composition of cells harvested from the dermis and epidermis of a skin tissue sample. The composition of cells has a cell population comprising keratinocytes basal cells, melanocytes, and fibroblasts; and the cell population of the composition and the tissue sample are comparable. Claims 29 and 61 also recite that the cell suspension is free of cellular congregates greater than 200 μM.

According to the Office action at pages 8 and 9, Hirobe 1991 teaches "a composition comprising cells dissociated form mouse whole skin tissue by cutting the tissue into small pieces and incubating the pieces tissue (sic) in a 0.25% solution of trypsin (page 185 under "Culture of melanocytes"). The composition of Hirobe is a suspension of single cells (*Id.*). and comprises a serum-free physiological saline…" The Office action further provides that "[i]n this case, this rejection might be overcome by a substantive evidentiary showing that the method steps recited in the cited claims produce a composition that is materially and patentably distinct from the skin cell suspension of Hirobe….The discussion of inherent properties in the rejection over Noel-Hudson also applies to this rejection for similar reasons." In addition, the Office action states that "[t]he composition of Hirobe is produced from a sample that has been enzymatically dissociated; therefore, the composition contains those cells that were present in the tissue sample. There is no disclosure in Hirobe that any cells have been destroyed, so the amount of each type

of cell relative to each other is comparable to that in the tissue; "comparable" is not synonymous with "identical"."

At page 185, column 1, under "Culture of melanocytes," Hirobe 1991 reports that skin tissue samples taken from mice were "cut into small pieces (5 X 5 mm^2) and incubated in 0.25% trypsin…in phosphate-buffered saline (PBS, pH 7.2) for 14-16 hours at 2° C. *Epidermal sheets were mechanically separated from the dermis with fine forceps* and floated onto 0.02% solution of ethylenediamine-tetra-acetate (EDTA…) in CMF-PBS in plastic centrifuge tubes." (Emphases added.) Hirobe 1991 goes on to report that the tubes were gently shaken and incubated at 37° C for 15 minutes. Afterwards, "the tissues were gently shaken repeatedly to produce a suspension of basal cells, and the cornified sheets were removed. Then the suspension of *epidermal cells* was gently and repeatedly introduced into and expelled from a Pasteur pipette to generate a suspension of single cells." (Emphasis added.) The cells were then pelleted by centrifugation and resuspended.

While Hirobe 1991 reports a suspension that includes single cells, nowhere does Hirobe 1991 teach or suggest, explicitly or inherently, that such suspension was free of all cellular congregates greater than 200 μM. Hirobe 1991 does not teach or suggest that the epidermal tissue or cells were subjected to any filtration or cell separation techniques beyond "gently" shaking, and "gently and repeatedly" pipetting the suspension in CMF-PBS with EDTA. One skilled in the art would readily appreciate that the method described by Hirobe 1991 would not inherently produce a suspension comprising only single cells in the absence of any cellular congregates greater than 200 μM. Furthermore, Hirobe 1991 completely fails to teach or suggest, explicitly or inherently, a cell suspension that has a composition of cells having a cell population comprising keratinocyte basal cells, melanocytes, ***and fibroblasts***—i.e., a composition cells harvested from both the epidermis (i.e., keratinocyte basal cells and melanocytes) ***and the dermis*** (i.e., fibroblasts). As such, Hirobe 1991 necessarily fails to teach or suggest a suspension having a cell composition that has a cell population that is comparable with the cell population with the tissue sample comprising dermis, epidermis and a dermal-epidermal junction therebetween. In fact, because Hirobe 1991 reports separating the epidermis from the dermis and producing a "suspension of epidermal cells" from the skin tissue sample,

Hirobe 1991 teaches away from the cell suspension of claims 29 and 61. As such Hirobe 1991 fails to teach each and every element of the instant claims.

In addition to the foregoing, the affidavit under 37 C.F.R. § 1.132 by Dr. Wood further emphasizes that Hirobe 1991's composition is neither identical nor substantially similar to Applicants' cell suspension. See, for example, paragraph 5C where Dr. Wood states that: "Hirobe at page 185 describes mechanically separating epidermal sheets from the dermis, preparing epidermal cells from the epidermal sheets, and gently and repeatedly pipetting the epidermal cells to produce a cell suspension. At least because the epidermis is separated from the dermis before any cell suspension is prepared, and because the cell suspension prepared by gentle pipetting is not free of cellular congregates greater than 200 μM, Hirobe's composition is different from U.S.S.N. 10/068,299. That is, at no time does Hirobe produce a composition that is either identical or substantially similar to the cell suspension of U.S.S.N. 10/068,299."

For at least the foregoing reasons, claims 29 and 61 are patentable over Hirobe 1991. Claims 63, 65, and 75-79 are dependent upon claim 61, and thus are also patentable over Hirobe 1991. Accordingly, Applicants respectfully request that the rejection of claims 29, 61, 63, 65, and 75-79 under 35 U.S.C. § 102(b) over Hirobe 1991 be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that the claims, as amended, are in condition for allowance and request favorable action. The Examiner is invited to contact Applicants' Attorney at the number below if in the Examiner's view it would expedite the prosecution of the application.

Respectfully submitted,

Date: April 29, 2010
Reg. No.: 43,526

Tel. No.: (617) 526-9841
Fax No.: (617) 526-9899

/Jennifer A. Camacho, Reg. No. 43,526/
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Exhibit D

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/068,299	02/06/2002	Fiona M. Wood	AVT-001	8540

42532 7590 07/09/2010

PROSKAUER ROSE LLP
ONE INTERNATIONAL PLACE
BOSTON, MA 02110

EXAMINER	
BARNHART, LORA ELIZABETH	
ART UNIT	**PAPER NUMBER**
1651	

MAIL DATE	DELIVERY MODE
07/09/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/068,299	WOOD ET AL.	
	Examiner	Art Unit	
	Lora E. Barnhart	1651	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *29 April 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *29,34-63,65 and 67-79* is/are pending in the application.

4a) Of the above claim(s) *34-60,62 and 67-74* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *29,61,63,65 and 75-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4/29/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendments

Applicant's amendments filed 4/29/10 to claims 29, 61, 65, 75, 76, 78, and 79 have been entered. No claims have been canceled or added in this reply. Claims 29, 34-63, 65, and 67-79 remain pending in the current application, of which claims 29, 61, 63, 65, and 75-79 are being considered on their merits. Claims 34-60, 62, and 67-74 remain withdrawn from consideration at this time. References not included with this Office action can be found in a prior action. Any rejections of record not particularly addressed below are withdrawn in light of the claim amendments and applicant's comments.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 29, 61, 63, and 75-79 are rejected under 35 U.S.C. 102(b) as being anticipated by Baur et al. (1997, WO 08/23602; reference N).

Baur teaches a cell suspension comprising melanocytes, keratinocytes, and fibroblasts in a serum-free medium. See page 23, lines 19-21; and page 15, lines 19-21. Baur's suspension is produced by obtaining skin tissue from human donors during surgery and floating the tissue in 0.5% trypsin "for a sufficient time to effect cell separation," for example at 37°C for about 30-60 minutes. See page 8, lines 11-31.

Baur characterizes the cells as “dissociated” and “separated," indicating that the suspension is a single-cell suspension. See page 8, line 30, and page 15, line 20. The cell ratios in Baur’s suspension are “comparable” to those in the skin tissue sample in the respect that it is possible to compare them to each other. The cells in Baur’s suspension are necessarily autologous to the patients from which the samples were taken.

The claims are product-by-process claims. Product-by-process claims are not necessarily limited by the steps in the claims. See M.P.E.P. § 2113. Accordingly, the only material requirement limiting the compositions in claim 29 and 61 is that they contain keratinocyte basal cells, melanocytes, and fibroblasts in a suspension lacking cellular congregates (e.g., a single-cell suspension) and further lacking xenogenic serum. The choice of starting material, isolation steps, choice of enzyme, and amount of enzyme do not affect the properties of the composition, absent evidence to the contrary. Baur’s composition contains all of the positively recited elements of applicants’ claims and lacks the necessarily excluded elements.

Claim 65 is rejected under 35 U.S.C. 102(b) as being anticipated by Baur et al. (1997, WO 08/23602) taken in light of Hart (2002, U.S. Patent 6.432,666; reference A).

The teachings of Baur are relied upon as above. Although Baur is silent as to the presence of Langerhans cells in the suspensions, Hart teaches that trypsinized skin suspensions contain Langerhans cells. See Example 1 at column 5. Therefore, Langerhans cells are inherently a component of Baur’s suspension.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 29, 61, 63, 65, and 75-79 are rejected under 35 U.S.C. 103(a) as being unpatentable over Baur et al. (1997, WO 08/23602) taken in view of Lucas et al. (1994, U.S. Patent 5,328,695; reference B) and Hart (2002, U.S. Patent 6.432,666).

Baur teaches a cell suspension comprising melanocytes, keratinocytes, and fibroblasts in a serum-free medium. See page 23, lines 19-21; and page 15, lines 19-21. Baur's suspension is produced by obtaining skin tissue from human donors during surgery and floating the tissue in 0.5% trypsin “for a sufficient time to effect cell separation,” for example at 37^{o}C for about 30-60 minutes. See page 8, lines 11-31. The cell ratios in Baur’s suspension are “comparable” to those in the skin tissue sample

in the respect that it is possible to compare them to each other. The cells in Baur's suspension are necessarily autologous to the patients from which the samples were taken.

Baur does not explicitly teach that large cell aggregates are absent from the suspension. Regarding claim 65, Baur does not explicitly teach that the suspension includes Langerhans cells.

Lucas teaches filtering cell suspensions, including skin suspensions, through 20μm filters to remove aggregates prior to culturing. See column 11, lines 20-28.

Hart teaches that trypsinized skin suspensions contain Langerhans cells. See Example 1 at column 5.

A person of ordinary skill in the art would have had a reasonable expectation of success in passing Hart's suspension through Lucas's filter because Lucas teaches that cells may pass through the filter and remain viable. The decision to remove aggregates would have constituted routine optimization, given that Lucas teaches that filtering aggregates out of cell suspensions before culturing the cells was known at the time of the invention.

The person of ordinary skill in the art would have had a further reasonable expectation of success that the suspension of Baur contains Langerhans cells because Hart teaches that trypsinization of skin, the process Baur used, yields a cell suspension that contains Langerhans cells. See M.P.E.P. § 2141.02, section V.

It would therefore have been obvious to a person of ordinary skill in the art at the time the invention was made to remove any large aggregates from Baur's suspension,

which Hart teaches includes Langerhans cells, using Lucas's filter prior to culturing Baur's cells because Lucas teaches and exemplifies doing so prior to culturing.

Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill at the time the invention was made.

Response to Arguments

Applicants' arguments in the 4/29/10 reply regarding the art rejections of record and the declaration made by inventor Fiona M. Wood under 37 C.F.R. 1.132 ("the Wood declaration") have been fully considered as they pertain to the new grounds of rejection, but they are not persuasive of error. The examiner believes that applicants' concerns have been fully addressed by the new Baur reference. The claim amendments require for the first time that the composition contain a population comprising keratinocyte basal cells, melanocytes, and fibroblasts; previously, all that was required was that the cells be viable. The new rejections were necessitated by the amendments to the independent claims.

Applicants allege that the term "comparable" should be interpreted as "similar." See reply, page 12, end of first paragraph. However, the term "comparable" is not explicitly provided with such a limiting definition, and in any case, the degree of "similarity" is not limited in the claims or the specification. The constituents in the suspension of Baur are necessarily comparable to some degree with the tissue that gave rise to that suspension. There is no evidence that the selection of dissociation method has a material effect on the properties of the claimed composition, e.g. that one manner of dissociation destroys or enriches a given cell type to such a degree that the

composition of suspension is not reasonably similar to the composition of the skin sample.

The examiner notes that in the working examples, applicants employ 0.5% trypsin at 37°C for 5-45 minutes to dissociate the skin sample, and Baur teaches incubating skin in 0.5% trypsin at 37°C for 30-60 minutes. Compare page 20, lines 17-19, of the instant specification with page 8, line 30, of Baur. Baur and the instant specification concur that the choice of incubation time may vary. See page 20, lines 19-22, of the instant specification with Baur's suggestion to incubate "for a sufficient time to effect cell separation." The examiner further notes that the working examples carry out steps that are encompassed by the steps recited in claims 29 and 61, but the examples contain no evidence that the suspension has any particular number of any particular cell type. If the ratio of cells within the suspension is critical, evidence to that effect (preferably, evidence comparing applicants' suspension to Baur's) would be probative.

Applicants' concerns at pages 14-19 about the presence of all of the cell types recited in the claims and the absence of congregates within the prior art suspensions are fully addressed in the above rejections.

No claims are allowed. No claims are free of the art.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lora E. Barnhart whose telephone number is (571)272-1928. The examiner can normally be reached on Monday-Thursday, 9:00am - 5:30pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G. Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Lora E Barnhart/
Primary Examiner, Art Unit 1651

Notice of References Cited	Application/Control No. 10/068,299	Applicant(s)/Patent Under Reexamination WOOD ET AL.	
	Examiner Lora E. Barnhart	Art Unit 1651	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,432,666	08-2002	Hart, Derek N.	435/69.1
*	B	US-5,328,695	07-1994	Lucas et al.	424/426
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	WO 97/23602	07-1997	WIPO	Baur et al.	C12N 5/08
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

SHEET 1 of 1

FORM PTO – 1449 SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT	ATTY DOCKET NO.: AVT-001 APPLICANTS: Wood et al. APPLICATION NO.: 10/068,299 FILING DATE: February 6, 2002 GROUP: 1651

U.S. PATENT DOCUMENTS

EXAM INIT.		DOCUMENT NO.	DATE	NAME	CLASS	SUB CLASS	FILING DATE IF APPROPRIATE

FOREIGN PATENT DOCUMENTS

EXAM INIT.		DOCUMENT NO.	DATE	COUNTRY CODE	CLASS	SUB CLASS	FILING DATE	ABSTRACT ONLY	ENGLISH LANG (Y/N)
	B1	39901/97	04/09/98	AU			10/03/97	N	Y

OTHER ART, JOURNAL ARTICLES, ETC.

EXAM INIT.		OTHER DOCUMENTS: (Including Author, Title, Date, Relevant Pages, Place of Publication)
	C9	Boyce, S.T., Ham, R.G., "Cultivation, frozen storage and clonal growth of normal human epithelial keratinocytes in serum-free media." *J. Tissue Cult. Methods* 9:83-93 (1985).
	C10	Navarro F. A., et al., "Sprayed keratinocyte suspensions accelerate epidermal coverage in a porcine microwound model." *J. Burn Care Rehabil.* 21:513-518 (2000).
	C11	Svensjö T., et al., "Autologous keratinocyte suspensions accelerate epidermal wound healing in pigs." *J. Surgical Res.* 99:211-221 (2001).
	C12	~~Product Number 352070 of BD e-Catalog: Centrifuges and test tubes, 3 pages, printed 5/03/2002~~
	C13	~~Product Number 352360 of BD e-Catalog: Centrifuges and test tubes, 2 pages, printed 5/03/2002~~
	C14	~~Kisker Biotech dry block heat and water baths product catalog, 3 pages, printed 5/03/2002~~
		no publication date

EXAMINER	/Lora E Barnhart/	DATE CONSIDERED	07/01/2010

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /leb/

Exhibit E

PATENT
Attorney Docket No.: 127630-010100

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Wood et al.	CONF. NO:	8540
APPLICATION NO.:	10/068,299	GROUP NO:	1651
FILING DATE:	February 6, 2002	EXAMINER:	Barnhart, Lora Elizabeth
TITLE:	CELL SUSPENSION PREPARATION TECHNIQUE AND DEVICE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR WITHDRAWAL OF FINALITY
AMENDMENT AND RESPONSE

Applicants submit this Request for Withdrawal of Finality and Amendment and Response in connection with the Final Office Action mailed July 9, 2010. Applicants also submit herewith an Information Disclosure Statement and respectfully request that the references cited therein be considered and made of record, upon withdrawal of the finality of the outstanding Office Action. The Commissioner is authorized to charge any fees, including the requisite fee under 37 C.F.R. § 1.17(p), occasioned by entry of this paper to Attorney's Deposit Account No. 50-2678.

Applicants respectfully request entry of this Request, in which:

- **Amendments to the Claims** begin on page 2;
- **Remarks** begin on page 10.

AMENDMENTS TO THE CLAIMS

This listing of the claims will replace all prior versions and listings of the claims in the application.

Listing of Claims

1-28. (Canceled)

29. (Currently amended) A cell suspension for immediate dispersion to a graft site on a patient produced according to a method comprising the steps of:

(a) physically and/or chemically dissociating cellular stratum in a split or full thickness skin tissue sample obtained from a patient ~~to provide cells suitable for grafting to the patient~~, wherein the split or full thickness skin tissue sample comprises dermis, epidermis, and a dermal-epidermal junction therebetween;

(b) harvesting cells from the dermis and the epidermis at the dermal-epidermal junction,

wherein the cells are harvested in the presence of a nutrient solution that is free of serum xenogenic to said patient and suitable for direct application to the graft site,

wherein the harvested cells comprise viable keratinocyte basal cells, melanocytes and fibroblasts autologous to the patient,

the harvested cells having the potential to include cellular congregates; and

(c) filtering the harvested cells in the nutrient solution to remove cellular congregates greater than 200 [[μM]]μm,

wherein the resulting cell suspension for immediate dispersion to the graft site comprises viable keratinocyte basal cells, melanocytes and fibroblasts harvested at the dermal-epidermal junction, and is free of serum xenogenic to said patient and free of cellular congregates greater than 200 [[μM]]μm~~, and~~

~~wherein the resulting cell suspension comprises a composition of viable cells autologous to said patient, and wherein said composition has a cell population comprising keratinocyte basal cells, melanocytes and fibroblasts, the cell population of the composition and the skin tissue sample being comparable~~.

30-33. (Canceled)

34. (Withdrawn) A cell suspension according to claim 29, the suspension being distributed on a patient tissue site undergoing tissue grafting.

35. (Withdrawn) A cell suspension according to claim 29 comprising the further step of:

(d) administering the suspension directly to a region on the patient that requires a cell graft.

36. (Withdrawn) A cell suspension according to claim 35 wherein the tissue sample is obtained from the patient that requires a cell graft.

37. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is distributed relatively evenly over the graft region.

38. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively.

39. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension contains cells present in a ratio to each other comparable to those in the donor sample.

40. (Withdrawn) A cell suspension according to claim 39 wherein the cells include keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

41. (Withdrawn) A suspension according to claim 40 wherein the cells are substantially viable.

42. (Withdrawn) A cell suspension according to claim 37 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

43. (Withdrawn) A cell suspension according to claim 35 wherein the cell suspension is obtained perioperatively from a tissue sample for the patient that requires a cell graft, contains

cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

44. (Withdrawn) A cell suspension produced according to a method comprising the steps of:

(a) obtaining a tissue sample from a site on a donor in need of a tissue graft;

(b) physically and/or chemically dissociating and removing cellular stratum from cells present in the sample;

(c) harvesting the cells in the presence of a nutrient solution;

(d) distributing the suspension on a site of the donor as an autologous tissue graft.

45. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of cell conglomerates.

46. (Withdrawn) A suspension according to claim 44 wherein the suspension is substantially free of xenogenic serum.

47. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is distributed relatively evenly over the graft region.

48. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is obtained perioperatively.

49. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension contains cells represent in a ratio to each other comparable to those seen in the donor sample.

50. (Withdrawn) A suspension according to claim 49 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

51. (Withdrawn) A suspension according to claim 50 wherein the cells are substantially viable.

52. (Withdrawn) A cell suspension according to claim 47 wherein the cell suspension is sprayed, spread, pipetted, or pained on to the tissue site.

53. (Withdrawn) A cell suspension according to claim 44 wherein the cell suspension is obtained perioperatively, contains cells present in a ratio to each other comparable to those seen in the donor sample, and is sprayed, spread, pipetted, or painted onto the tissue site to provide an even distribution over the graft region.

54. (Withdrawn) A cell suspension produced by a method comprising obtaining cells from a patient in need of a tissue graft, providing the cells in nutrient solution in a manner that is substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension being distributed in apposition to the site of the recipient as a tissue graft.

55. (Withdrawn) A suspension according to claim 54 wherein the suspension is distributed relatively evenly over the graft region.

56. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension is obtained perioperatively.

57. (Withdrawn) A cell suspension according to claim 54 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

58. (Withdrawn) A suspension according to claim 57 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

59. (Withdrawn) A suspension according to claim 58 wherein the cells are substantially viable.

60. (Withdrawn) A cell suspension according to claim 55 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

61. (Currently amended) A cell suspension for immediate dispersion to a graft site on a patient, comprising cells harvested from a split or full thickness skin tissue sample obtained from [[a]]the patient, wherein the split or full thickness skin tissue sample comprises dermis, epidermis, and a dermal-epidermal junction therebetween, the cell suspension comprising:

(a) ~~a composition of~~ viable cells harvested from the dermis and the epidermis at the dermal-epidermal junction ~~of said skin tissue sample~~ and autologous to said patient, said ~~composition having a cell population~~ viable cells comprising keratinocyte basal cells, fibroblasts and melanocytes~~, the cell population of the composition and the skin tissue sample being comparable~~; and

(b) a nutrient solution free of serum xenogenic to the patient and suitable for direct application to the graft site on the patient,

wherein said cell suspension for immediate dispersion to the graft site comprises viable keratinocyte basal cells, melanocytes and fibroblasts harvested at the dermal-epidermal junction, and is free of cellular congregates greater than 200 μm.

62. (Withdrawn) A suspension according to claim 61 wherein the suspension is distributed relatively evenly over the graft region.

63. (Previously presented) A cell suspension according to claim 61 wherein the cell suspension is obtained perioperatively.

64. (Canceled)

65. (Previously presented) A suspension according to claim 61 wherein said composition of cells comprises keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

66. (Canceled)

67. (Withdrawn) A cell suspension according to claim 62, wherein the cell suspension is sprayed, spread pipetted, or painted onto the tissue site.

68. (Withdrawn) A cell suspension produced by a method sufficient to provide the cells in nutrient solution, substantially free of cellular stratum, xenogenic serum, and cell conglomerates, the suspension serving as a graft in apposition to the body of a recipient in need of a tissue graft.

69. (Withdrawn) A suspension according to claim 68 wherein the suspension is distributed relatively evenly over the graft region.

70. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is obtained perioperatively.

71. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension contains cells present in a ratio to each other comparable to those seen in the donor sample.

72. (Withdrawn) A suspension according to claim 71 wherein the cells comprise keratinocyte basal cells, Langerhans cells, fibroblasts and melanocytes.

73. (Withdrawn) A suspension according to claim 72 wherein the cells are substantially viable.

74. (Withdrawn) A cell suspension according to claim 69 wherein the cell suspension is sprayed, spread, pipetted, or painted onto the tissue site.

75. (Previously presented) A cell suspension according to claim 61, wherein the viable cells are harvested in a method using a solution comprising an enzyme.

76. (Previously presented) A cell suspension according to claim 75 wherein the enzyme is selected from the group consisting of trypsin, trypsin-EDTA, dispase, collagenase, thermolysin, pronase, hyaluronidase, pancreatin, elastase, and papain.

77. (Previously presented) A cell suspension according to claim 76 wherein the enzyme is trypsin.

78. (Previously presented) A cell suspension according to claim 77 wherein the trypsin is present in the solution in an amount that is between 5 and 0.1% per volume of the solution.

79. (Previously presented) A cell suspension according to claim 77 wherein the trypsin is present in the solution in an amount that is less than 0.05% per volume of the solution.

80. (New) A cell suspension according to claim 29 wherein harvesting comprises scraping cells from a surface at the dermal-epidermal junction.

81. (New) A cell suspension according to claim 29 wherein the nutrient solution comprises a physiological saline.

82. (New) A cell suspension according to claim 29 wherein the resulting cell suspension for immediate dispersion to the graft site is immediately dispersed to the graft site without *in vitro* culturing.

83. (New) A cell suspension according to claim 29 wherein a concentration of the resulting cell suspension for immediate dispersion to the graft site is modified before dispersion to the graft site.

84. (New) A cell suspension according to claim 61 wherein the viable cells are harvested by scraping cells from a surface at the dermal-epidermal junction.

85. (New) A cell suspension according to claim 61 wherein the nutrient solution comprises a physiological saline.

86. (New) A cell suspension according to claim 61 wherein said cell suspension for immediate dispersion to the graft site is immediately dispersed to the graft site without *in vitro* culturing.

87. (New) A cell suspension according to claim 61 wherein a concentration of said cell suspension for immediate dispersion to the graft site is modified before dispersion to the graft site.

REMARKS

Request for Withdrawal of Finality

The outstanding Office Action was made final. MPEP § 706.07(a) specifies the conditions under which the finality of a second or subsequent Office Action is proper, providing that:

> "Under present practice, second or any subsequent actions on the merits shall be final, except where the examiner introduces a new ground of rejection that is neither necessitated by applicant's amendment of the claims nor based on information submitted in an information disclosure statement…."

Applicants respectfully submit that the finality of the outstanding Office Action is improper under MPEP § 706.07(a) because the Examiner introduces new grounds of rejection that are neither necessitated by Applicants' amendments nor based on an information disclosure statement. As such, the finality is premature and thus withdrawal of the finality pursuant to MPEP § 706.07(d) is respectfully requested.

The Examiner stated that "[t]he claim amendments require for the first time that the composition contain a population comprising keratinocyte basal cells, melanocytes, and fibroblasts; previously, all that was required was that the cells be viable. The new rejections were necessitated by the amendments to the independent claims." Office Action at page 6, emphasis added. Applicants respectfully disagree. The recitation of keratinocyte basal cells, melanocytes, and fibroblasts was not presented for the first time in the Amendment and Response filed April 29, 2010. In contrast, the recitation was presented in the preceding Amendment and Response filed October 19, 2009. Specifically, the relevant prosecution history is as follows:

1. In the Amendment and Response filed October 19, 2009, the independent claims recite "said composition has a ratio of keratinocyte basal cells, melanocytes, and fibroblasts that is comparable to…."
2. In the non-final Office Action dated December 29, 2009, the Examiner requested clarification of "ratio" (see page 4).

3. In the Amendment and Response filed April 29, 2010, Applicants amended the claims to remove "ratio" and to recite "said composition has a cell population comprising keratinocyte basal cells, melanocytes, and fibroblasts."
4. In the final Office Action dated July 9, 2010, the Examiner alleges that the claims "require for the first time" keratinocyte basal cells, melanocytes, and fibroblasts (see page 6).

From the above prosecution history, it is clear that the recitation of keratinocyte basal cells, melanocytes, and fibroblasts was previously presented for examination (i.e., in the preceding Office Action dated December 29, 2009). Thus, the recitation cannot be reasonably said to be presented for the first time in the outstanding Office Action.

Furthermore, it is clear that in the preceding Office Action dated December 29, 2009, the Examiner understood that the claims required at least the three cell types (i.e., keratinocyte basal cells, melanocytes, and fibroblasts). Specifically, the Examiner cited Van Bossuyt to support the position that skin contains keratinocytes, melanocytes, and fibroblasts, and that Noel-Hudson's biopsy contains "all of the cell types recited in claims 29, 61, and 65." See Office Action dated December 29, 2009 at page 6. Therefore, the Examiner has previously considered as a claim limitation that the claimed composition includes keratinocyte basal cells, melanocytes, and fibroblasts (i.e., in the preceding Office Action dated December 29, 2009). Thus, this claim limitation cannot be reasonably said to be considered for the first time in the outstanding Office Action.

At least because the recitation of keratinocyte basal cells, melanocytes, and fibroblasts was not presented for the first time, Applicants respectfully submit that the new grounds of rejection made in the final Office Action are neither necessitated by a claim amendment nor an information disclosure statement. As such, the conditions set forth in MPEP § 706.07(a) have not been satisfied. Accordingly, Applicants respectfully request the finality of the final Office Action be reconsidered and withdrawn. Entry of the present Amendment and Response is also requested.

In the Claims

Claims 29, 61, 63, 65, and 75-79 were considered in the Office Action of July 9, 2010. Claims 34-60, 62, and 67-74 stand withdrawn from consideration. Claims 29, 61, 63, 65, and 75-79 stand rejected.

Applicants hereby amend claims 29 and 61 for clarity. Claim 29 has also been amended to correct typographical errors (i.e., to replace "μM" with "μm"). New claims 80-87 have been added. The amendments to claims 29 and 61 are supported by the originally filed claims and specification (e.g., page 7, line 10; page 21, line 11; page 22, line 11; page 5, lines 14-16; page 8, lines 20-21; and page 11, line 25). Support for new claims 80 and 83 can be found, for example, in the originally filed specification at page 22, lines 10-13. Support for new claims 81 and 85 can be found, for example, in the originally filed specification at page 11, lines 1-6. Support for new claims 82 and 86 can be found, for example, in the originally filed specification at page 8, lines 3-6 and page 12, lines 20-23. Support for new claims 83 and 87 can be found, for example, in the originally filed specification at page 12, lines 7-9 and page 4, lines 7-9. No new matter is introduced by these amendments.

Applicants have amended certain claims solely to expedite prosecution of the application. In making these amendments, Applicants are not acquiescing to the pending rejections and are not abandoning or surrendering any of the subject matter in previous versions or listings of the claims or in the application. Accordingly, Applicants reserve the right to pursue claims of similar, narrower, or broader scope in the future.

In view of the amendments to the claims and the following remarks, Applicants respectfully request reconsideration and withdrawal of all grounds of rejection.

Rejection Under 35 U.S.C. § 102(b)

Claims 29, 61, 63, and 75-79

Claims 29, 61, 63, and 75-79 stand rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by WO 97/23602 by Baur et al. ("Baur"). Applicants respectfully traverse this basis of rejection because Baur does not disclose every claimed element as amended.

Independent claims 29 and 61, as amended, recite that the cell suspension for immediate dispersion to the graft site includes viable keratinocyte basal cells, melanocytes and fibroblasts

harvested at the dermal-epidermal junction, and that the nutrient solution is suitable for direct application to the graft site. The Examiner noted on page 8 of the September 23, 2008 Final Office Action that "[l]imitations such as 'a cell suspension ... suitable for direct application to a region on a patient undergoing tissue grafting' are statements of intended use; see M.P.E.P. § 2111.02." Applicants respectfully submit that M.P.E.P. § 2111.02 concerns the effect of preamble and thus is not applicable to Applicants' claims as amended, because the amended claims recite structural or functional limitations such as "for immediate dispersion to the graft site," "harvested at the dermal-epidermal junction," and "suitable for direct application to the graft site."

It should also be noted that certain limitations provided in the amended claims, although may be functional in nature, such functional language does not render a claim improper. *In re Swinehart*, 439 F.2d 210, 169 USPQ 226 (CCPA 1971). As clearly provided in M.P.E. P. § 2173.05(g), "[a] functional limitation must be evaluated and considered, just like any other limitation of the claim, for what it fairly conveys to a person of ordinary skill in the pertinent art in the context in which it is used. A functional limitation is often used in association with an element, ingredient or step of a process to define a particular capability or purpose that is served by the recited element, ingredient or step."

Applicants respectfully submit that Baur does not anticipate claims 29 and 61 as amended because Baur does not disclose every claimed element. First, Baur does not disclose a cell suspension for immediate dispersion to the graft site that includes viable keratinocyte basal cells, melanocytes and fibroblasts. Instead, Baur discloses a cell culture for skin grafting that does not include fibroblasts. In particular, Baur produces "primary keratinocyte or melanocytes produced under serum free conditions without the use of any feeder cells, said primary keratinocytes and melanocytes being used for skin grafting." Baur at page 5, lines 13-15, emphasis added. Further, Baur provides that feeder cells include fibroblasts by stating " 'feeder cells' (e.g., fibroblasts)." See Baur at page 5, line 11. Thus, one of ordinary skill in the art would understand that Baur does not include fibroblasts in its cell culture for skin grafting.

The Examiner's position is that Baur teaches a cell suspension comprising melanocytes, keratinocytes, and fibroblasts. Office Action at page 2. However, Baur's cell suspension is not for immediate dispersion to the graft site. Rather, Baur obtains and prepares a skin sample "such that it is suitable for culturing in vitro." Baur at page 8, lines 18-19, emphasis added. The cells

from the skin sample are cultured and expanded, where "the expanded primary melanocytes or keratynocytes may be used prior to immortalization, e.g., in skin grafting." Baur at page 16, lines 6-7, emphasis added. Specifically, Baur provides that, "a cell suspension produced from skin samples described in example 1, which contains dissociated melanocytes, keratinocytes and fibroblasts, are cultured in the subject NR-3 medium." Baur at page 23, lines 19-21.

Second, Baur fails to disclose a cell suspension comprising cells harvested at the dermal-epidermal junction. Rather, Baur separates the dermis and epidermis, and prepares cells from the dermis and epidermis separately. Indeed, Baur from page 8, line 25 to page 9, line 2 and page 21, lines 21-27 provides:

> The resultant skin sections are then preferably separated into dermis and epidermis. This may be effected by physical and/or enzymatic means. For example, this may be effected by trypsinization, e.g. by floating skin sheets in a trypsin solution (e.g. about 0.5%) containing EDTA (e.g. about 0.1%) for a sufficient time to effect cell separation, e.g. about 30-60 minutes at 37°C or overnight at 4°C.
>
> The dermis is separated (to isolate the fibroblasts, see EXAMPLE 2) and the epidermis is then placed in a suspension medium. Preferably the suspension medium will contain soybean trypsin inhibitor solution (SBTI) and will be contacted with the cells for a sufficient time, typically about 5 minutes, in order to inactivate the trypsin and provide for cell release. Tissue culture medium will then be added, preferably serum-free NR-2 medium (described infra) and a filter (e.g. 100 mm filter) to obtain the desired cells, i.e. keratinocytes and/or melanocytes.
>
> Human fibroblasts were isolated from the skin samples FKO-NR, GKO- NR, DKO-NR. After the separation of the dermal and epidermal compartment the dermis was cut into small pieces 0.2 x 0.2 mm and fixed on a 6cm culture plate with serum. Dulbecco's minimal essential medium (DMEM, 10% FCS) was added after 2-4 hours. This explant culture was then incubated until fibroblast outgrowth was visible. Confluent fibroblast cultures were split and expanded for frozen stocks.

Thus, it is clear that Baur independently isolates cells from the entire dermal layer and from the entire epidermal layer, as opposed to the dermal-epidermal junction.

The Examiner took the position that the claims are product-by-process claims and "are not necessarily limited by the steps in the claims. See M.P.E.P § 2113." Office Action at page 3. However, M.P.E.P § 2113 also provides that "[t]he structure implied by the process steps should be considered when assessing the patentability of product-by-process claims over the prior art, especially where the product can only be defined by the process steps by which the

product is made, or where the manufacturing process steps would be expected to impart distinctive structural characteristics to the final product. See, e.g., *In re Garnero*, 412 F.2d 276, 279, 162 USPQ 221, 223 (CCPA 1979) (holding 'interbonded by interfusion' to limit structure of the claimed composite and noting that terms such as 'welded,' 'intermixed,' 'ground in place,' 'press fitted,' and 'etched' are capable of construction as structural limitations.)." Following the same line of reasoning, "harvested at the dermal-epidermal junction" is capable of construction as a structural limitation, because the process step (e.g., choice of the dermal-epidermal junction as the starting material) imparts distinctive structural characteristics to the final composition.

The Examiner also alleges that "Baur teaches a cell suspension… in a serum-free medium." Office Action at page 2. However, while Baur may produce melanocytes and keratinocytes in a serum-free medium, it does not produce fibroblasts under serum-free conditions. Rather, Baur prepares fibroblasts in the presence of serum. Specifically, Baur states that "[h]uman fibroblasts were isolated from the skin samples FKO-NR, GKO- NR, DKO-NR. After the separation of the dermal and epidermal compartment the dermis was cut into small pieces 0.2 x 0.2 mm and fixed on a 6cm culture plate with serum. Dulbecco's minimal essential medium (DMEM, 10% FCS) was added after 2-4 hours." Baur at page 21, lines 21-25, emphases added. In contrast, Applicants harvest cells in a nutrient solution that is free of serum xenogenic to the patient.

Third, Baur does not disclose a nutrient solution suitable for direct application to the graft site. Rather, Baur teaches tissue culture media for in vitro culturing. Baur at page 4, lines 31-33 and page 8, line 19. Baur is silent with regard to direct application of the tissue culture media to skin graft site.

For at least the foregoing reasons, claims 29 and 61 are patentable over Baur. Claims 63 and 75-79 are dependent upon claim 61, and thus are also patentable over Baur. Accordingly, Applicants respectfully request that the rejection of claims 29, 61, 63, and 75-79 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

Claim 65

Claim 65 stands rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Baur in view of U.S. Patent No. 6,432,666 to Hart ("Hart"). Applicants respectfully traverse this basis of rejection because Baur in view of Hart does not disclose every claimed element as amended.

Claim 65 depends upon claim 61. Claim 61 as amended is patentable over Baur for reasons discussed above. Hart does not remedy the deficiencies of Baur at least because Hart is cited solely as teaching that trypsinized skin suspensions contain Langerhans cells. See Office Action at page 3.

For at least the foregoing reasons, claim 61 and its dependent claim 65 are patentable over Baur in view of Hart. Accordingly, Applicants respectfully request that the rejection of claim 65 under 35 U.S.C. § 102(b) be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 103(a)

Claims 29, 61, 63, 65, and 75-79 stand rejected under 35 U.S.C. § 103(a) as allegedly being anticipated by Baur in view of U.S. Patent No. 5,328,695 to Lucas et al. ("Lucas") and Hart. Applicants respectfully traverse this basis of rejection because Baur in view of Lucas and Hart does not teach, suggest, or make obvious every claimed element as amended.

Independent claims 29 and 61, as amended, recite that the cell suspension for immediate dispersion to the graft site includes viable keratinocyte basal cells, melanocytes and fibroblasts harvested at the dermal-epidermal junction, and that the nutrient solution is suitable for direct application to the graft site. Baur, Lucas, and Hart do not teach or suggest these elements, nor would it have been obvious to one of ordinary skill in the art to modify the cited art to produce claims 29 and 61.

As discussed above, Baur does not teach or suggest a cell suspension for immediate dispersion to the graft site that includes viable keratinocyte basal cells, melanocytes and fibroblasts. Instead, Baur prepares cells "without 'feeder cells' (e.g., fibroblasts)" and thus teaches excluding fibroblasts. Baur, *supra*. Furthermore, Baur's mere mentioning of skin grafting relates only to keratinocytes and melanocytes and excludes fibroblasts. Baur, *supra*. In addition, Baur teaches in vitro culturing of keratinocytes and melanocytes prior to skin grafting. Baur, *supra*. As such, Baur explicitly teaches away from Applicants' claimed cell suspension that is for immediate dispersion.

Baur also fails to teach or suggest a cell suspension harvested at the dermal-epidermal junction. Rather, Baur harvests cells from separated dermis and epidermis separately and independently. Baur, *supra*. Baur uses the entire dermis and epidermis, as opposed to specific

compartment of the skin (i.e., the dermal-epidermal junction as Applicants' claims require). Baur, *supra*. Even if Baur may independently harvest different cell types, and then combine the cells in one suspension, the resulting cell composition would still be inherently distinct from those harvested at the dermal-epidermal junction. Indeed, one of ordinary skill in the art would understand that such contrasting methods necessarily result in distinctive structural characteristics to the final cell composition.

Baur's cell culture is inherently distinct from Applicants' claimed cell suspension for the additional reason that Baur offers the opposite, incompatible teaching regarding cell harvesting methods. Baur provides different methods for dermal and epidermal cell isolation. In particular, Baur teaches that melanocytes and keratinocytes are produced in a serum-free medium, while fibroblasts are prepared in the presence of serum. Baur, *supra*. By contrast, Applicants' claimed cell suspension includes cells (including keratinocyte basal cells, melanocytes and fibroblasts) that are harvested in a nutrient solution that is free of serum xenogenic to the patient.

Furthermore, Baur does not teach or suggest a nutrient solution suitable for direct application to the graft site. Rather, Baur's media are for in vitro culturing. Baur, *supra*. One of ordinary skill in the art would understand that culture media generally include macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, sugar, solidifying agents or support systems, and growth regulators. Indeed, Baur at pages 32-34 provides a list of ingredients that are rich in amino acids, vitamins, and other organic supplements. Because culture media are rich in nutrients, they are generally handled with care in highly sterile environments (e.g., in a sterile hood) to avoid microbial contamination. Given that Baur's culture media are rich in nutrients and susceptible to pathogen contamination, one of ordinary skill in the art would not consider them suitable for direct application to a graft site.

Lucas and Hart do not cure the deficiencies of Baur. Rather, Lucas is cited solely as teaching filtering cell suspensions and Hart is cited solely as teaching that trypsinized skin suspensions contain Langerhans cells. See Office Action at page 5. Thus, the combination of the references still fails to teach, suggest, or make obvious claims 29 and 61, as well as their dependent claims.

For at least the foregoing reasons, claims 29 and 61 are patentable over Baur in view of Lucas and Hart. Claims 63, 65, and 75-79 are dependent upon claim 61, and thus are also

patentable. Accordingly, Applicants respectfully request that the rejection of claims 29, 61, 63, 65, and 75-79 under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully submit that the claims, as amended, are in condition for allowance and request early favorable action. If the Examiner believes a telephonic interview would expedite the prosecution of the present application, the Examiner is welcome to contact Applicants' Attorney at the number below.

Respectfully submitted,

Date: September 9, 2010
Reg. No.: 43,526
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/Jennifer A. Camacho, Reg. No. 43,526/
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Exhibit F

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/068,299	02/06/2002	Fiona M. Wood	127630-010100/US	8540

35893 7590 09/22/2010

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EXAMINER	
BARNHART, LORA ELIZABETH	

ART UNIT	PAPER NUMBER
1651	

NOTIFICATION DATE	DELIVERY MODE
09/22/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

BosIpMail@gtlaw.com
santosv@gtlaw.com
vinsonl@gtlaw.com

Continuation Sheet for Advisory Action

Continuation of Box 3. The proposed amendments will not be entered because they introduce numerous new limitations that have not been considered or searched by the examiner, e.g. “for immediate dispersion to a graft site on a patient” and “split or full thickness skin tissue sample.” These limitations would require more than the cursory review permitted after final rejection. The amendments also propose to add four new claims without canceling at least four finally rejected claims.

Continuation of Box 11. The request for reconsideration has been fully considered, but it does NOT place the application in condition for allowance.

As an initial matter, the finality of the 7/9/10 Office action was proper because although the words "keratinocyte basal cells, melanocytes, and fibroblasts" appeared in the 10/19/09 claims, those claims did not actually require the sample to contain all three types of cells. The 10/19/09 claims required that the composition comprise a ratio of these cells “comparable” to the ratio found in a skin sample, but the claims set forth no basis for comparison and did not actually require that the skin sample be one that contained all three. The 4/29/10 amendments require the composition to comprise these three cell types, not just to have “a ratio” of the cell types that is somehow “comparable” to the “ratio” in another sample. For further discussion of the limitation in question, see the 12/30/09 Office action at page 4 (rejecting the claim because the limitations did not place metes and bounds on the composition). In the interest of compact prosecution, the examiner granted applicant the courtesy of interpreting the claims favorably for art

rejection purposes. (12/30/09 Office action, page 5.) By applicants' rule, an examiner complying with the Office's policy favoring compact prosecution could never make an Office action final.

The 9/9/10 claims have not been entered, but the remarks submitted therewith are not persuasive of error. Applicant alleges that M.P.E.P. § 2111.02 regards the preamble and therefore "is not applicable to applicants' claims," which is confusing because the intended use "for immediate dispersion to a graft site" in claim 29 indeed appears in the preamble. Such functional limitations do not affect the structure and physical properties of the composition. While describing a product in terms of its function is not itself improper (see *In re Swinehart*, 439 F.2d 210, 169USPQ 226 (CCPA 1971)), claims directed to a product should be distinguished from the prior art product in terms of structure rather than function; this point was recently revisited. "When a claim limitation is defined in purely functional terms, the task of determining whether that limitation is sufficiently definite is a difficult one that is highly dependent on context (e.g., the disclosure in the specification and the knowledge of a person of ordinary skill in the relevant art area). We note that the patent drafter is in the best position to resolve the ambiguity in the patent claims, and it is highly desirable that patent examiners demand that applicants do so in appropriate circumstances so that the patent can be amended during prosecution rather than attempting to resolve the ambiguity in litigation." *Halliburton Energy Services, Inc. v. M-I LLC*, 85 USPQ2d 1654, 1663 (Fed. Cir. 2008). While functional claiming is authorized by 35 U.S.C. § 112, sixth paragraph, that statute was enacted specifically to preclude overly broad claims that effectively purport to cover

any and all limitations, so long as they perform the required functions. Specifically, claims that are ambiguous as to boundaries for functional limitations may be indefinite and do not distinguish the claimed product over the prior art. It is not clear which cell suspensions made using the steps of claim 29 would be suitable "for immediate dispersion to a graft site on a patient" and which would not. For this reason, the intended use limitation has no clear patentable weight. Applicant appears to be attempting to claim within the product claim a method of using that product, which is improper. Applicant's comments about the handling of *in vitro* samples relative to those intended for application at page 17 are not supported by evidence, and there is no clear relationship between a propensity toward "pathogen contamination" and the claimed composition. If applicants' composition has reduced susceptibility toward contamination, the claims should so recite in a manner that limits the structure.

Applicant's arguments about the dermal-epidermal junction are not clearly relevant to the patentability of the claimed composition. The claims require beginning with a tissue sample that contains dermis, epidermis, and the junction (step (a)), but this is an inherent property of skin; in fact, step (b) recited in claim 29 only requires harvesting cells from the dermis and the epidermis. The "wherein" limitations at the end of claim 29 provide the only structural requirements for the claimed composition: The lack of xenogenic serum, a particular maximum congregate size, and a requirement for three types of cells to be present. Applicant has provided no evidence that any of the steps are critical to obtaining a composition with those properties.

Furthermore, applicant's arguments about Baur's teachings appear to be based on an assumption that Baur has discarded the dermal-epidermal junction, but this is not positively recited in the reference and not supported by evidence. Baur teaches separating the dermis from the epidermis, then processing the portions. There is no clear reason to presuppose that the junction is not included in at least one of these samples. The absence of a statement that Baur's method retains the junction cannot be interpreted as evidence that the junction must necessarily have been discarded. Applicant's statement at page 17 that Baur's cell composition "would still be inherently different from those [cells] harvested at the dermal-epidermal junction" has no basis in evidence, and in any case, the claims do not require such a harvest. This point also addresses applicants' argument about product-by-process limitations -- applicant has provided no evidence that the steps recited in claim 29 yield a product materially different from that of Baur. See M.P.E.P. § 2113.

Applicants' argument about Baur's use of serum are not germane to the rejection of record because Baur's NR-3 medium is "fully defined," i.e. free of serum. See page 22, line 5. Therefore, Baur's Example 3 teaches a serum-free suspension of cells. As above, there is no evidence that the manner in which the cells are obtained has any material effect on the resulting composition. Applicant has provided no comparison between cells isolated using serum-free medium and then further cultured in that medium and cells isolated using serum-free medium and then further cultured in serum-free medium. See M.P.E.P. § 2113.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lora E. Barnhart whose telephone number is (571)272-1928. The examiner can normally be reached on Monday-Thursday, 9:00am - 5:30pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Michael G. Wityshyn can be reached on 571-272-0926. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Lora E Barnhart/
Primary Examiner, Art Unit 1651

Advisory Action Before the Filing of an Appeal Brief	**Application No.** 10/068,299	**Applicant(s)** WOOD ET AL.	
	Examiner Lora E. Barnhart	**Art Unit** 1651	

--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

THE REPLY FILED 09 September 2010 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE.

1. ☒ The reply was filed after a final rejection, but prior to or on the same day as filing a Notice of Appeal. To avoid abandonment of this application, applicant must timely file one of the following replies: (1) an amendment, affidavit, or other evidence, which places the application in condition for allowance; (2) a Notice of Appeal (with appeal fee) in compliance with 37 CFR 41.31; or (3) a Request for Continued Examination (RCE) in compliance with 37 CFR 1.114. The reply must be filed within one of the following time periods:
   a) ☐ The period for reply expires _____months from the mailing date of the final rejection.
   b) ☒ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.

   Examiner Note: If box 1 is checked, check either box (a) or (b). ONLY CHECK BOX (b) WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

NOTICE OF APPEAL

2. ☐ The Notice of Appeal was filed on _____. A brief in compliance with 37 CFR 41.37 must be filed within two months of the date of filing the Notice of Appeal (37 CFR 41.37(a)), or any extension thereof (37 CFR 41.37(e)), to avoid dismissal of the appeal. Since a Notice of Appeal has been filed, any reply must be filed within the time period set forth in 37 CFR 41.37(a).

AMENDMENTS

3. ☒ The proposed amendment(s) filed after a final rejection, but prior to the date of filing a brief, will not be entered because
   (a) ☒ They raise new issues that would require further consideration and/or search (see NOTE below);
   (b) ☒ They raise the issue of new matter (see NOTE below);
   (c) ☒ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or
   (d) ☒ They present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: *see continuation sheet*. (See 37 CFR 1.116 and 41.33(a)).

4. ☐ The amendments are not in compliance with 37 CFR 1.121. See attached Notice of Non-Compliant Amendment (PTOL-324).
5. ☐ Applicant's reply has overcome the following rejection(s): _____.
6. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).
7. ☐ For purposes of appeal, the proposed amendment(s): a) ☐ will not be entered, or b) ☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.
   The status of the claim(s) is (or will be) as follows:
   Claim(s) allowed: _____.
   Claim(s) objected to: _____.
   Claim(s) rejected: _____.
   Claim(s) withdrawn from consideration: _____.

AFFIDAVIT OR OTHER EVIDENCE

8. ☐ The affidavit or other evidence filed after a final action, but before or on the date of filing a Notice of Appeal will not be entered because applicant failed to provide a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See 37 CFR 1.116(e).
9. ☐ The affidavit or other evidence filed after the date of filing a Notice of Appeal, but prior to the date of filing a brief, will not be entered because the affidavit or other evidence failed to overcome all rejections under appeal and/or appellant fails to provide a showing a good and sufficient reasons why it is necessary and was not earlier presented. See 37 CFR 41.33(d)(1).
10. ☐ The affidavit or other evidence is entered. An explanation of the status of the claims after entry is below or attached.

REQUEST FOR RECONSIDERATION/OTHER

11. ☒ The request for reconsideration has been considered but does NOT place the application in condition for allowance because: see continuation sheet.
12. ☐ Note the attached Information *Disclosure Statement*(s). (PTO/SB/08) Paper No(s). _____
13. ☐ Other: _____.

/Lora E Barnhart/
Primary Examiner, Art Unit 1651